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(54) Title: FLOW DEVICES FOR THE REDUCTION OF COMPOUNDS FROM BIOLOGICAL COMPOSITIONS AND METHODS OF USE

(57) Abstract

Methods and devices are provided for reducing the concentration of low molecular weight compounds in a biological composition, while substantially maintaining a desired biological activity of the biological composition. The device comprises highly porous adsorbent particles, and the adsorbent particles are immobilized by an inert matrix.

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# FLOW DEVICES FOR THE REDUCTION OF COMPOUNDS FROM BIOLOGICAL COMPOSITIONS AND METHODS OF USE

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of copending application Serial Number 09/003,113, filed January 6, 1998, which is incorporated by reference in its entirety.

## TECHNICAL FIELD

The present invention relates to methods and devices for the reduction of compounds from biological compositions. The compounds have a molecular weight ranging from about 100 g/mol to about 30,000 g/mol.

## BACKGROUND ART

An extensive body of research exists regarding the removal of substances from blood products. The bulk of this research is directed at white cell reduction. *See, e.g.*, M.N. Boomgaard *et al.*, *Transfusion* 34:311 (1994); F. Bertolini *et al.*, *Vox Sang* 62:82 (1992); and A.M. Joustra-Dijkhuis *et al.*, *Vox Sang* 67:22 (1994). Filtration of platelets is the most common method used in white cell reduction of platelet concentrates. *See, e.g.*, M. Böck *et al.*, *Transfusion* 31:333 (1991) (Sepacell PL-5A, Asahi, Tokyo, Japan); J.D. Sweeney *et al.*, *Transfusion* 35:131 (1995) (Leukotrap PL, Miles Inc., Covina, CA); and M. van Marwijk *et al.*, *Transfusion* 30:34 (1990) (Cellselect, NPBI, Emmer-Compascuum, The Netherlands; Immugard Ig-500, Terumo, Tokyo, Japan). These current filtration mechanisms, however are not amenable for the removal of relatively low molecular weight compounds including for example psoralens, psoralen photoproducts and other compounds commonly used in treating biological fluids.

The process of adsorption has been used to isolate selective blood components onto phospholipid polymers. For example, several copolymers with various electrical charges have been evaluated for their interactions with blood components, including platelet adhesion and protein adsorption. K. Ishihara *et al.*,

*J. Biomed. Mat. Res.* 28:1347 (1994). Such polymers, however, are not designed for the adsorption of low molecular weight compounds.

Various dialysis means are able to remove low molecular weight compounds from plasma and whole blood. For example, dialysis can successfully remove low molecular weight toxins and pharmaceutical compounds. Thus, dialysis might be used to remove, for example, psoralens and psoralen photoproducts from blood products. Unfortunately, current dialysis procedures involve very complicated and expensive devices. As such, the use of dialysis machines would not be practical for the decontamination of a large volume of blood products.

The use of polystyrene divinylbenzene, silica gel, and acrylester polymers for the adsorption of methylene blue has previously been described. For example, PCT Publication No. WO 91/03933 describes batch studies with free adsorbent resin (*e.g.*, Amberlites (Rohm and Haas (Frankfurt, Germany) and Bio Beads (Bio-Rad Laboratories (Munich, Germany)). Without very careful removal of the adsorbent resins after exposure to the blood product, however, these methods create the risk of transfusion of the resin particles.

In addition, devices and processes for the removal of leukocytes and viral inactivation agents (*e.g.*, psoralens, hypericin, and dyes such as methylene blue, toluidine blue, and crystal violet) have also been disclosed. Specifically, PCT Publication No. WO 95/18665 describes a filter comprising a laid textile web which includes a mechanically stable polymeric substrate. The web itself comprises interlocked textile fibers forming a matrix with spaces and fibrillated particles disposed within the spaces. However, this device causes a significant decrease in the Factor XI activity.

Simpler, safer and more economical means for reducing the concentration of low molecular weight compounds in a biological composition while substantially maintaining the biological activity of the treated composition are therefore needed.

## DISCLOSURE OF THE INVENTION

Devices are provided for reduction of concentration of compounds from biological compositions. The compounds have molecular weights ranging from about 100 g/mol to about 30,000 g/mol. The device is a flow device. An example of a flow device is shown in Figure 12. Flow devices are known in the literature and are described, for example, in PCT publication WO 96/40857, incorporated by reference herein. Flow devices permit reduction of concentration of low molecular weight compounds from materials such as blood products by perfusing the blood product through the flow device.

Exemplary compounds include pathogen inactivating compounds, dyes, thiols, plasticizers and activated complement. Devices are provided that comprise a three dimensional network of adsorbent particles immobilized by an inert matrix. This immobilization reduces the risk of leakage of loose adsorbent particles into the blood product. Furthermore, immobilization of the adsorbent particles by an inert matrix simplifies manufacturing by reducing problems associated with handling loose adsorbent particles.

The present invention provides a method of reducing the concentration of a biological response modifier in a biological composition, wherein the method substantially maintains a desired biological activity of the biological composition. The method comprises treating the biological composition with a device.

In one embodiment, the device comprises an inert matrix containing highly adsorbent particles, wherein the adsorbent particles range from about 1 μm to about 200 μm in diameter, and wherein the device is for use in a flow process.

In another embodiment, the biological response modifier is activated complement.

In another embodiment, the biological composition is plasma.

In another embodiment, the adsorbent material has a length less than three times the width.

In another embodiment, the adsorbent particles comprise a hypercrosslinked polystyrene network.

In another embodiment, the adsorbent particles are activated carbon particles, wherein the activated carbon particles have a surface area greater than about 1200 $m^2/g$.

In another embodiment, the activated carbon particles are formed by steam activation of coconut shells.

In another embodiment, the inert matrix of the device is a fiber network composed of cellulose.

In another embodiment, the inert matrix is a particulate network formed by sintering together particles of ultra-high molecular weight polyethylene with particles of hypercrosslinked polystyrene networks.

In another embodiment, the method further reduces the concentration of a psoralen derivative, an acridine derivative, a dye or a quencher in the biological composition.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 diagrammatically depicts a perspective view of one embodiment of a fiber, indicating its inner core and outer sheath, that forms the fiber networks of the fiberized resin.

FIG. 2 schematically represents a portion of one embodiment of the fiberized resin of the present invention.

FIG. 3 diagrammatically represents a cross-sectional view of one embodiment of fiberized resin in which the adsorbent beads are secured to fibers that make up the fiberized resin.

FIG. 4 diagrammatically represents a cross-sectional view of one embodiment of fiberized resin in which the adsorbent beads are immobilized within the fibers of the fiberized resin and the heat seals that encompass samples of fiberized resin.

FIG. 5 is a graph showing a comparison of adsorption kinetics for removal of aminopsoralens from platelets with Dowex® XUS-43493 and Amberlite®

XAD-16 HP loose adsorbent beads and fiberized resin containing Amberlite® XAD-16.

FIG. 6 is a graph showing a comparison of the adsorption kinetics for removal of aminopsoralens from platelets with p(HEMA)-coated and uncoated Dowex® XUS-43493 beads.

FIG. 7 is a graph showing a comparison of the effect of pre-treatment solution glycerol content on relative aminopsoralens adsorption capacity for Amberlite® XAD-16 and Dowex® XUS-43493.

FIG. 8 is a graph showing a comparison of the effect of wetting solution on 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen adsorption capacities for dried adsorbent in 100% plasma for Amberlite® XAD-16 (bottom) and Dowex® XUS-43493 (top); the samples that were not wet in an ethanol solution are labeled "No Tx". Adsorbent capacities are reported as percentages relative to the capacity of optimally wet adsorbent.

FIG. 9 is a graph showing a comparison of adsorption of aminopsoralens over a 3-hour period from plasma using Amberlite® XAD-16 wet in several different solutions.

FIG. 10 is a graph showing a comparison of the kinetics of adsorption of methylene blue over a 2-hour period from plasma.

FIG. 11 depicts the chemical structures of acridine, acridine orange, 9-amino acridine, and 5-[(β-carboxyethyl)amino]acridine.

FIG. 12 is an illustration of a flow configuration for the immobilized adsorption device (IAD).

FIG. 13 is an illustration of an experimental set-up for a whole blood perfusion study.

FIG. 14 shows an exploded view of a disk configuration assembly made according to the invention.

FIG. 15 shows an exploded view of a drip chamber assembly made according to the invention.

## BEST MODE FOR CARRYING OUT THE INVENTION

Devices are provided for reduction of concentration of compounds from biological compositions. The compounds have molecular weights ranging from about 100 g/mol to about 30,000 g/mol. The device is a flow device. An example of a flow device is shown in Figure 12. Flow devices are known in the literature and are described, for example, in PCT publication WO 96/40857, incorporated by reference herein. Flow devices permit reduction of concentration of low molecular weight compounds from materials such as blood products by perfusing the blood product through the flow device.

Exemplary compounds include pathogen inactivating compounds, dyes, thiols, plasticizers and activated complement. Devices are provided that comprise a three dimensional network of adsorbent particles immobilized by an inert matrix. This immobilization reduces the risk of leakage of loose adsorbent particles into the blood product. Furthermore, immobilization of the adsorbent particles by an inert matrix simplifies manufacturing by reducing problems associated with handling loose adsorbent particles.

*Definitions*

The term "acridine derivatives" refer to a chemical compound containing the tricyclic structure of acridine (dibenzo[*b*,*e*]pyridine; 10-azanthracene). The compounds have an affinity for (and can bind) to nucleic acids non-covalently through intercalation. The term "aminoacridine" refers to those acridine compounds with one or more nitrogen-containing functional groups. Examples of aminoacridines include 9-amino acridine and acridine orange (depicted in Figure 11).

The term "adsorbent particle" broadly refers to any natural or synthetic material which is capable of interacting with molecules in a liquid thus allowing the molecule to be removed from the liquid. Examples of naturally occurring adsorbents include but are not limited to activated carbon, silica, diatomaceous earth, and cellulose. Examples of synthetic adsorbents include but are not limited to polystyrene, polyacrylics, and carbonaceous adsorbents. Adsorbent particles are often porous, often possess high surface areas, and may be modified with a

variety of functional groups (e.g. ionic, hydrophobic, acidic, basic) which can effect how the adsorbent interacts with molecules.

The term "aromatic," "aromatic compounds," and the like refer broadly to compounds with rings of atoms having delocalized electrons. The monocyclic compound benzene ($C_6H_6$) is a common aromatic compound. However, electron delocalization can occur over more than one adjacent ring (*e.g.*, naphthalene (two rings) and anthracene (three rings)). Different classes of aromatic compounds include, but are not limited to, aromatic halides (aryl halides), aromatic heterocyclic compounds, aromatic hydrocarbons (arenes), and aromatic nitro compounds (aryl nitro compounds).

The term "biocompatible coating" refers broadly to the covering of a surface (*e.g.*, the surface of a polystyrene bead) with a hydrophilic polymer that when in contact with a blood product does not result in an injurious, toxic, or immunological response and renders the surface more biocompatible by decreasing cell adhesion, protein adsorption or improves cell function. Suitable coatings are biocompatible if they have minimal, if any, effect on the biological material to be exposed to them. By "minimal" effect it is meant that no significant biological difference is seen compared to the control. In preferred embodiments, biocompatible coatings improve the surface hemocompatibility of polymeric structures. For example, poly(2-hydroxyethyl methacrylate) (pHEMA) is frequently used for the coating of materials used in medical devices (*e.g.*, blood filters).

The term "biocompatible housing" refers broadly to filter housings, containers, bags, vessels, receptacles, and the like that are suitable for containing a biological material, such as plasma. Suitable containers are biocompatible if they have minimal, if any, effect on the biological material to be contained therein. By "minimal" effect it is meant that no significant biological difference is seen in blood product function compared to the control as described herein, for example, for plasma. Thus, biological compositions may be stored in biocompatible housings prior to transfusion to a recipient.

The term " biological fluids" include human or non-human whole blood, plasma, platelets, red blood cells, leukocytes, serum, lymph, saliva, milk, urine, or products derived from or containing any of the above, alone or in mixture, with or without a chemical additive solution. Preferably, the fluid is blood or a blood product with or without a chemical additive solution, most preferably plasma.

The term "blood bag" refers to a blood product container.

The term "blood product" refers to the fluid and/or associated cellular elements and the like (such as erythrocytes, leukocytes, platelets, etc.) that pass through the body's circulatory system; blood products include, but are not limited to, blood cells, platelet mixtures, serum, and plasma. The term "platelet mixture" refers to one type of blood product wherein the cellular element is primarily or only platelets. A platelet concentrate (PC) is one type of platelet mixture where the platelets are associated with a smaller than normal portion of plasma. In blood products synthetic media may make up that volume normally occupied by plasma; for example, a platelet concentrate may entail platelets suspended in 35% plasma/65% synthetic media. Frequently, the synthetic media comprises phosphate.

The term "blood separation means" refers broadly to a device, machine, or the like that is able to separate blood into blood products (*e.g.*, platelets and plasma). An apheresis system is one type of blood separation means. Apheresis systems generally comprise a blood separation device, an intricate network of tubing and filters, collection bags, an anticoagulant, and a computerized means of controlling all of the components.

The term "crosslinked" refers broadly to linear molecules that are attached to each other to form a two- or three-dimensional network. For example, divinylbenzene (DVB) serves as the crosslinking agent in the formation of styrene-divinylbenzene copolymers. The term also encompasses "hypercrosslinking" in which hypercrosslinked networks are produced by crosslinking linear polystyrene chains either in solution or in a swollen state with bifunctional agents. A variety of bifunctional agents can be used for cross-linking

(for example, see Davankov and Tsyurupa, Reactive Polymers 13:24-42 (1990); Tsyurupa et al., Reactive Polymers 25:69-78 (1995).

The term "cyclic compounds" refers to compounds having one (*i.e.*, a monocyclic compounds) or more than one (*i.e.*, polycyclic compounds) ring of atoms. The term is not limited to compounds with rings containing a particular number of atoms. While most cyclic compounds contain rings with five or six atoms, rings with other numbers of atoms (*e.g.*, three or four atoms) are also contemplated by the present invention. The identity of the atoms in the rings is not limited, though the atoms are usually predominantly carbon atoms. Generally speaking, the rings of polycyclic compounds are adjacent to one another; however, the term "polycyclic" compound includes those compounds containing multiple rings that are not adjacent to each other.

The term "dye" refers broadly to compounds that impart color. Dyes generally comprise chromophore and auxochrome groups attached to one or more cyclic compounds. The color is due to the chromophore, while the dying affinities are due to the auxochrome. Dyes have been grouped into many categories, including the azin dyes (*e.g.*, neutral red, safranin, and azocarmine B); the azo dyes; the azocarmine dyes; the dephenymethane dyes; the fluorescein dyes; the ketonimine dyes; the rosanilin dyes; the triphenylmethane dyes; the phthalocyanines; and, hypericin. It is contemplated that the methods and devices of the present invention may be practiced in conjunction with any dye that is a cyclic compound.

The term "fiberized resin" generally refers to immobilization of adsorbent material, including for example, resins, in, on or entrapped to a fiber network. In one embodiment, the fiber network is comprised of polymer fibers. In another embodiment, the fibers consist of a polymer core (*e.g.*, polyethylene terephthalates [PET]) with a high melting point surrounded by a polymer sheath (*e.g.*, nylon or modified PET) with a relatively low melting temperature. Fiberized resin may be produced by heating the fiber network, under conditions that do not adversely affect the adsorbent capacity of the resin to a significant degree. Where the resin comprises beads, heating is performed such that the

adsorbent beads become attached to the outer polymer sheath to create "fiberized beads". By producing fiberized resin containing a known amount of adsorbent beads per defined area, samples of fiberized resin for use in the removal of cyclic compounds (*e.g.*, psoralens, and, in particular, aminopsoralens) and other products can be obtained by cutting a defined area of the fiberized resin, rather than weighing the adsorbent beads.

The term "filter" refers broadly to devices, materials, and the like that are able to allow certain components of a mixture to pass through while retaining other components. For example, a filter may comprise a mesh with pores sized to allow a blood product (*e.g.*, plasma) to pass through, while retaining other components such as resin particles. The term "filter" is not limited to the means by which certain components are retained.

The term "flow adapter" refers to a device that is capable of controlling the flow of a particular substance like a blood product. The flow adapter may perform additional functions, such as preventing the passage of pieces of adsorbent resin material.

The term "heterocyclic compounds" refers broadly to cyclic compounds wherein one or more of the rings contains more than one type of atom. In general, carbon represents the predominant atom, while the other atoms include, for example, nitrogen, sulfur, and oxygen. Examples of heterocyclic compounds include furan, pyrrole, thiophene, and pyridine.

The phrase "high temperature activation process" refers to a high temperature process that typically results in changes in surface area, porosity and surface chemistry of the treated material due to pyrolysis and/or oxidation of the starting material.

The term "immobilized adsorption device (IAD)" refers to immobilized adsorbent material in, on or entrapped to an inert matrix. Where the inert matrix is a fiber network the term IAD can be used interchangeably with the term fiberized resin.

The term "inert matrix" refers to any synthetic or naturally occurring fiber or fibrous material which can be used to immobilize adsorbent particles without

substantially effecting the desired biological activity of the blood product. The matrix may contribute to the reduction in concentration of small organic compounds although typically it does not contribute substantially to the adsorption or removal process. In addition, the inert matrix may interact with cellular or protein components resulting in cell removal (e.g. leukodepletion) or removal of protein or other molecules. The matrix may undergo a surface treatment or coating to enhance functionality. For example, the matrix may get a hydrophobic coating or glow discharge treatment to increase biocompatibility, increase wettability, and/or facilitate priming.

The term "in-line column" refers to a container, usually cylindrically shaped, having an input end and an output end and containing a substance disposed therein to reduce the concentration of small organic compounds from a blood product.

The term "isolating" refers to separating a substance out of a mixture containing more than one component. For example, platelets may be separated from whole blood. The product that is isolated does not necessarily refer to the complete separation of that product from other components.

The term "macropores" generally means that the diameter of the pores is greater than about 500 Å. The term micropores refers to pores with diameters less than about 20 Å. The term mesopores refers to pores with diameters greater than about 20 Å. and less than about 500 Å.

The term "macroporous" is used to describe a porous structure having a substantial number of pores with diameters greater than about 500 Å.

The term "macroreticular" is a relative term that means that the structure has a high physical porosity (*i.e.*, a large number of pores are present) a porous adsorbent structure possessing both macropores and micropores.

The term "mesh enclosure," "mesh pouch" and the like refer to an enclosure, pouch, bag or the like manufactured to contain multiple openings. For example, the present invention contemplates a pouch, containing the immobilized adsorbent particle, with pores of a size that allow a blood product to contact the

immobilized adsorbent particle, but retain the immobilized adsorbent particle within the pouch.

The term "photoproduct" refers to products that result from the photochemical reaction that a psoralen or other dye (e.g., methylene blue, phthalocyanines) undergoes upon exposure to ultraviolet radiation.

The term "polyaromatic compounds" refers to polymeric compounds containing aromatic groups in the backbone, such as polyethylene terphalate, or as pendant groups, such as polystyrene, or both.

The term "polystyrene network" refers broadly to polymers containing styrene ($C_6H_5CH=CH_2$) monomers; the polymers may be linear, consisting of a single covalent alkane chain with phenyl substituents, or cross-linked, generally with *m*- or *p*-phenylene residues or other bifunctional or hypercrosslinked structure, to form a two-dimensional polymer backbone.

The term "psoralen removal means" refers to a substance or device that is able to remove greater than about 80% of the psoralen from, *e.g.*, a blood product; preferably, greater than about 90%; most preferably greater than about 99%. A psoralen removal means may also remove other components of the blood product, such as psoralen photoproducts.

The phrase "reducing the concentration" refers to the removal of some portion of low molecular weight compounds from a biological composition. While reduction in concentration is preferably on the order of greater than about 70%, more preferably on the order of about 90%, and most preferably on the order of about 99%.

The phrase "removing substantially all of said portion of a compound (*e.g.* a psoralen, psoralen derivative, isopsoralen, acridine, acridine derivative, or dye) free in solution" refers preferably to the removal of more than about 80% of the compound free in solution, more preferably to the removal of more than about 85%, even more preferably of more than about 90%, and most preferably to the removal of more than about 99%.

The term "resin" refers to a solid support (such as particles or beads etc.) capable of interacting and attaching to various small organic compounds,

including psoralens, in a solution or fluid (*e.g.*, a blood product), thereby decreasing the concentration of those elements in solution. The removal process is not limited to any particular mechanism. For example, a psoralen may be removed by hydrophobic or ionic interaction (*i.e.*, affinity interaction). The term "adsorbent resin" refers broadly to both natural organic substances and synthetic substances and to mixtures thereof.

The term "sintered medium" refers to a structure which is formed by applying heat and pressure to a powder or mixture of powders, thereby partially fusing the powder or powder mixture, such that a path for a flowing fluid exists through the structure. The porous structure can be prepared by mixing powders of relatively low melting polymers and heating them so the plastic particles partially fuse but still allow a path for fluids to penetrate the porous mass. Sintered adsorbent media can be prepared similarly by incorporating carbon or other high or non-melting adsorbent particle with that of the low melting powder and heating. Methods of producing porous plastic materials are described in U.S. Patent Nos. 3,975,481, 4,110,391, 4,460,530, 4,880,843 and 4,925,880, incorporated by reference herein. The process causes fusing of the powder particles resulting in the formation of a porous solid structure. The sintered medium can be formed into a variety of shapes by placing the polymeric powder in a forming tool during the sintering process. Adsorbent particles can be introduced into the sintered medium by mixing adsorbent particles with the powdered thermoplastic polymer before subjecting to the sintering process.

The term "stabilizing agent" refers to a compound or composition capable of optimizing the adsorption capacity of certain resins. Generally speaking, acceptable stabilizing agents should be soluble in water and ethanol (or other wetting agents), nonvolatile relative to water and ethanol, and safe for transfusion in small amounts. Examples of stabilizing agents include, but are not limited to, glycerol and low molecular weight PEGs. A "wetting agent" is distinguishable from a "stabilizing agent" in that the former is believed to reopen adsorbent pores of those resins that are not hypercrosslinked (*i.e.*, non-macronet resins). Wetting agents generally will not prevent pores from collapsing under drying conditions,

whereas stabilizing agents will. A general discussion of wetting and wetting agents is set forth in U.S. Patent No. 5,501,795 to Pall *et al.*, hereby incorporated by reference.

The phrase "substantially maintaining a desired biological activity of the biological composition" refers to substantially maintaining properties of a biological composition which are believed to be indicative of the potential performance of the composition in a therapeutic setting. For example, in the case of plasma, *in vivo* activity is not destroyed or significantly lowered if the level of clotting factors, such as Factors I, II, V, VII, VIII, IX, X, XI, or the change in PT and PTT time are substantially maintained in plasma when treated by the methods described herein. For example, the change in level of clotting factors, such as Factors I, II, V, VII, VIII, IX, X, XI of the treated plasma can be less than about 20%; preferably less than about 10%. The change in PT and PTT time for the treated plasma can be, for example, less than about 3 seconds and greater than 1 second; preferably 1.5 seconds. It is further contemplated that the phrase substantially maintained for each of the properties associated with a described biological composition may also include values acceptable to those of ordinary skill in the art as described in the literature, including for example in Klein H.G., ed. Standards for Blood Banks and Transfusion Services, 17$^{th}$ Ed., Bethesda, MD: American Association of Blood Banks, 1996, incorporated by reference herein.

The term "equivalent thereto" when used in reference to a device of the present invention refers to a device that functions equivalently with respect to the maintenance of biological activity of a biological composition. For example, an "equivalent" device or matrix containing adsorbent particles is one that similarly maintains a suitable coagulation factor level.

The term "low molecular weight compound" refers to an organic or biological molecule having a molecular weight ranging from about 100 g/mol to about 30,000 g/mol. Low molecular weight compounds include, without limitation, the following compounds: small organic compounds such as psoralens, acridines or dyes; quenchers, such as glutathione; plastic extractables, such as plasticizers; biological modifiers, such as activated complement, that

possess a molecular weight between about 100 g/mol and about 30,000 g/mol; and, polyamine derivatives.

The term "biological composition that is suitable for infusion" refers to a biological composition that maintains its essential biological properties (e.g. clotting function in plasma) while having sufficiently low levels of any undesired compounds (e.g. inactivation compounds, response modifiers) such that infusion provides intended function without detrimental side effects.

The term "control," as used in phrases such as "relative to control," refers to an experiment performed to study the relative effects of different conditions. For example, where a biological composition is treated with a device, "untreated control" would refer to the biological compostion in the absence of treatment with the device. It may also refer to a comparison between two different types of devices.

The term "4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen" is alternatively referred to as "S-59."

The term "N-(9-acridinyl)-β-alanine" is alternatively referred to as "5-[(β-carboxyethyl)amino]acridine." It is further alternatively referred to as "S-300."

The term "XUS-43493" is alternatively referred to as "Optipore 493."

The term "non-fibrous adsorbent material" refers to an adsorbent material composed substantially of particles that have a length, or longest dimension, less than five times their width, or narrowest dimension.

*Adsorbent Particles*

Provided are adsorbent particles which are useful in a device for reducing the concentration of compounds in a biological composition while substantially maintaining a desired biological activity of the biological composition. Typically, the compounds that are reduced in the biological composition have molecular weights ranging from about 100 g/mol to about 30,000 g/mol.

The adsorbent particles can be of any regular or irregular shape that lends itself to incorporation into the inert matrix but are preferably roughly spherical. The particles are greater than about 1 μm in diameter; preferably, the particles are greater than about 10 μm in diameter when using a sintered medium as the matrix

for the adsorbent, more preferably, the particles are between about 50 μm and about 150 μm in diameter when using a sintered medium as the matrix. Preferably the particles are between about 1 μm and about 200 μm in diameter when using a wet laid fibrous medium as the matrix, more preferably between about 1 μm and about 50 μm when using a wet laid fibrous medium as the matrix for the adsorbent.

In one preferred embodiment the adsorbent particles are activated carbons derived either from natural or synthetic sources. Nonlimiting examples of activated carbons include; Picatiff Medicinal, which is available from PICA USA Inc. (Columbus, OH), Norit ROX 0.8, which is available from Norit Americas, Inc. (Atlanta, GA), Ambersorb 572, which is available from Rohm & Haas (Philadelphia, PA), and G-277, which is available from PICA (Columbus, OH).

Preferred activated carbons are those that are specially cleaned and/or meet United States Pharmacopoeia Standards. Moreover, activated carbons with surface areas greater than 950 m2/g are preferred and those with surface areas greater than 1200 m2/g are more preferred, as activated carbons with more surface area available to the low molecular weight compound generally show better adsorption. Activated carbons formed by steam activation tend to have more hydrophobic surfaces, so for more hydrophobic low molecular weight compounds, these steam activated carbons often have better binding capacity and these carbons are preferred. Less macroporosity confers selectivity for low molecular weight compounds over large proteins that mediate biological activity, so activated carbons with less macroporosity, for example activated carbons prepared from coconut shell are also preferred.

In one preferred embodiment the particles are Norit A Supra, which is available from Norit Americas, Inc. (Atlanta, GA). Norit A Supra is a USP-grade activated carbon that is formed by steam activation of coconut shell. This activated carbon has a very high total surface area (2000 $m^2/g$) and is very microporous in nature.

In another preferred embodiment, the particles can be hydrophobic resins. Nonlimiting examples of hydrophobic resins include the following polyaromatic

adsorbents: Amberlite® adsorbents (*e.g.*, Amberlite® XAD-2, XAD-4, and XAD-16), available from Rohm and Haas (Philadelphia, PA); Amberchrom® adsorbents available from Toso Haas (TosoHass, Montgomeryville, PA); Diaion®//Sepabeads® Adsorbents (*e.g.*, Diaion® HP20), available from Mitsubishi Chemical America, Inc. (White Plains, NY); Hypersol-Macronet® Sorbent Resins (*e.g.*, Hypersol-Macronet® Sorbent Resins MN-200, MN-150 and MN-400) available from Purolite (Bala Cynwyd, PA); and Dowex® Adsorbents (*e.g.*, Dowex® XUS-40323, XUS-43493, and XUS-40285), available from Dow Chemical Company (Midland, MI).

Preferred particles are hydrophobic resins which are polyaromatic adsorbents comprising a hypercrosslinked polystyrene network, such as Dowex® XUS-43493 (known commercially as Optipore® L493 or V493) and Purolite MN-200.

Hypercrosslinked polystyrene networks, such as Dowex® XUS-43493 and Purolite MN-200 are non-ionic macroporous and macroreticular resins. The non-ionic macroreticular and macroporous Dowex® XUS-43493 has a high affinity for psoralens, including for example, 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen, and it possesses superior wetting properties. The phrase "superior wetting properties" means that dry (*i.e.* essentially anhydrous) adsorbent does not need to be wet with a wetting agent (*e.g.*, ethanol) prior to being contacted with the blood product in order for the adsorbent to effectively reduce the concentration of small organic compounds from the blood product.

Hypercrosslinked polystyrene networks, such as Dowex® XUS-43493 and Purolite MN-200 are preferably in the form of spherical particles with a diameter range of about 10 μm to about 200 μm. Adsorbent particles, including for example, Dowex® XUS-43493, preferably have extremely high internal surface areas and relatively small pores (*e.g.* 46 Å). The internal surface area of the particle can be from about 300 to about 1100 $m^2/g$; preferably 1100 $m^2/g$. The pores size of the particle can be greater than 25Å and less than 800Å; preferably from about 25Å to about 150Å; most preferably from about 25Å to about 50Å. While it is not intended that the present invention be limited to the mechanism by

which reduction of small organic compounds takes place, hydrophobic interaction is believed to be the primary mechanism of adsorption. Its porous nature confers selectively on the adsorption process by allowing small molecules to access a greater proportion of the surface area relative to large molecules (*i.e.*, proteins) and cells. Purolite® has many similar characteristics to Dowex® XUS-43493, such as high affinity for psoralens and superior wetting properties, and is also a preferred adsorbent particle.

Polystyrene particles can be classified, based on their mechanism of synthesis and physical and functional characteristics, as i) conventional networks and ii) hypercrosslinked networks. Preferred adsorbents have a high surface area, have pores that do not collapse, and do not require wetting. In addition, preferred adsorbents have low levels of extractable residual monomer, crosslinkers and other organic extractables.

The conventional networks are primarily styrene-divinylbenzene copolymers in which divinylbenzene (DVB) serves as the crosslinking agent (*i.e.*, the agent that links linear polystyrene chains together). These polymeric networks include the "gel-type" polymers. The gel-type polymers are homogeneous, non-porous styrene-DVB copolymers obtained by copolymerization of monomers. The macroporous adsorbents represent a second class of conventional networks. They are obtained by copolymerization of monomers in the presence of diluents that precipitate the growing polystyrene chains. The polystyrene network formed by this procedure possess a relatively large internal surface area (up to hundreds of square meters per gram of polymer); Amberlite® XAD-4 is produced by such a procedure.

In contrast to the conventional networks described above, the preferred adsorbents of the present invention (*e.g.*, Dowex® XUS-43493) are hypercrosslinked networks. These networks are produced by crosslinking linear polystyrene chains either in solution or in a swollen state with bifunctional agents; the preferred bifunctional agents produce conformationally-restricted crosslinking bridges, that are believed to prevent the pores from collapsing when the adsorbent is in an essentially anhydrous (*i.e.*, "dry") state.

The hypercrosslinked networks are believed to possess three primary characteristics that distinguish them from the conventional networks. First, there is a low density of polymer chains because of the bridges that hold the polystyrene chains apart. As a result, the adsorbents generally have a relatively large porous surface area and pore diameter. Second, the networks are able to swell; that is, the volume of the polymer phase increases when it contacts organic molecules. Finally, the hypercrosslinked polymers are "strained" when in the dry state; that is, the rigidity of the network in the dry state prevents chain-to-chain attractions. However, the strains relax when the adsorbent is wetted, which increases the network's ability to swell in liquid media. Davankov and Tsyurupa, *Reactive Polymers* 13:27-42 (1990); Tsyurupa *et al.*, *Reactive Polymers* 25:69-78 (1995), hereby incorporated by reference..

Several cross-linking agents have been successfully employed to produce the bridges between polystyrene chains, including *p*-xylene dichloride (XDC), monochlorodimethyl ether (MCDE), 1,4-bis-chloromethyldiphenyl (CMDP), 4,4'-bis-(chloromethyl)biphenyl (CMB), dimethylformal (DMF), *p,p'*-bis-chloromethyl-1,4-diphenylbutane (DPB), and tris-(chloromethyl)-mesitylene (CMM). The bridges are formed between polystyrene chains by reacting one of these cross-linking agents with the styrene phenyl rings by means of a Friedel-Crafts reaction. Thus, the resulting bridges link styrene phenol rings present on two different polystyrene chains. *See, e.g.*, U.S. Patent No. 3,729,457, hereby incorporated by reference.

The bridges are especially important because they generally eliminate the need for a "wetting" agent. That is, the bridges prevent the pores from collapsing when the adsorbent is in an essentially anhydrous (*i.e.*, "dry") state, and thus they do not have to be "reopened" with a wetting agent prior to the adsorbent being contacted with a blood product. In order to prevent the pores from collapsing, conformationally-restricted bridges should be formed. Some bifunctional agents like DPB do not result in generally limited conformation; for example, DPB contains four successive methylene units that are susceptible to conformation

rearrangements. Thus, DPB is not a preferred bifunctional agent for use with the present invention.

Some of the structurally-related characteristics of the above-described adsorbent particles are summarized in Table A.

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**TABLE A**

| Resin | Chemical Nature | Mean Surface Area ($m^2/g$) | Mean Pore Diam. (Å) | Mesh Size (µm) |
|---|---|---|---|---|
| **Amberlite® Adsorbents - Rohm and Haas** | | | | |
| XAD-2 | polyaromatic | 300 | 90 | 20-60 |
| XAD-4 | polyaromatic | 725 | 40 | 20-60 |
| XAD-7 | polymethacrylate | 450 | 90 | 20-60 |
| XAD-16 | polyaromatic | 800 | 100 | 20-60 |
| XAD-1180 | polyaromatic | 600 | 300 | 20-60 |
| XAD-2000 | polyaromatic | 580 | 42 | 20-60 |
| XAD-2010 | polyaromatic | 660 | 280 | 20-60 |
| **Amberchrom® Adsorbents - Toso Haas** | | | | |
| CG-71m | polymethacrylate | 450-550 | 200-300 | 50-100 |
| CG-71c | polymethacrylate | 450-550 | 200-300 | 80-160 |
| CG-161m | polyaromatic | 800-950 | 110-175 | 50-100 |
| CG-161c | polyaromatic | 800-950 | 110-175 | 80-160 |
| **Diaion®//Sepabeads® Adsorbents - Mitsubishi Chemical** | | | | |
| HP20 | polyaromatic | 500 | 300-600 | 20-60 |
| SP206 | brominated styrenic | 550 | 200-800 | 20-60 |
| SP207 | brominated styrenic | 650 | 100-300 | 20-60 |
| SP850 | polyaromatic | 1000 | 50-100 | 20-60 |
| HP2MG | polymethacrylate | 500 | 200-800 | 25-50 |
| HP20SS | polyaromatic | 500 | 300-600 | 75-150 |
| SP20MS | polyaromatic | 500 | 300-600 | 50-100 |
| **Dowex® Adsorbents - Dow Chemical Company** | | | | |
| XUS-40285 | functionalized | 800 | 25 | 20-50 |
| XUS-40323 | polyaromatic | 650 | 100 | 16-50 |

TABLE A

| | | | | |
|---|---|---|---|---|
| XUS-43493 | polyaromatic | 1100 | 46 | 20-50 |

*Processing the Adsorbent Particles*

The adsorbent particles may be further processed to remove fine particles, salts, potential extractables, and endotoxin. The removal of these extractable components is typically performed by treatment with either organic solvents, steam, or supercritical fluids. Preferably the particles are sterilized.

Several companies currently sell "cleaned" (*i.e.*, processed) versions of commercially available adsorbent particles. In addition to processing the adsorbent particles (*e.g.* resins), these companies test the adsorbents, and the final adsorbent is certified sterile (USP XXI), pyrogen-free (LAL), and free of detectable extractables (DVB and total organics).

Thermal processing (*e.g.*, steam) is an effective method for processing adsorbent particles. F. Rodriguez, *Principles Of Polymer Systems*, (Hemisphere Publishing Corp.), pp. 449-53 (3rd. Ed., 1989). Supelco, Inc. (Bellefonte, PA) uses a non-solvent, thermal proprietary process to clean the Dowex® XUS-43493 and Amberlite adsorbents. The main advantage of using steam is that it does not add any potential extractables to the adsorbent. One big disadvantage, however, is that this process can strip water from the pores of the resin beads; effective performance of some adsorbents requires that the beads be re-wet prior to contacting the illuminated blood product.

*The Use of Wetting Agents and Stabilizing Agents with Adsorbent Resins*

Methods may be used for preventing drying and loss of adsorption capacity of particles, such as Amberlite® which lose some of their adsorption capacity under certain conditions (*e.g.*, drying).

In one method, particles, materials or devices may be manufactured in a wet state which is sealed and not capable of drying. This method is associated with several important drawbacks. The shelf-life of the products could be reduced since levels of extractables from the materials could increase over time. Sterilization may be limited to a steam process because γ-irradiation of wet

polymers is typically not performed. Manufacturing a device that requires that a component be maintained in a wet state is, in general, more difficult than manufacturing a dry device; for example, bioburden and endotoxin may become of concern if there is a long lag time between device assembly and terminal sterilization.

A second method for preventing loss of adsorption capacity involves the use of an adsorbent which is not adversely affected by drying. As previously set forth, macroreticular adsorbents possessing highly crosslinked porous structures (*e.g.*, Dowex® XUS-43493 and Purolite® MN-200) generally do not require a wetting agent because the crosslinks prevent the pores from collapsing. Unlike Amberlite® XAD-16, these macroreticular adsorbents retain a very high proportion of their initial activity when they are dried.

In a third method, loss of adsorption capacity upon drying may be prevented by hydrating Amberlite® XAD-16 and related adsorbents (*e.g.*, Amberlite® XAD-4) in the presence of a non-volatile wetting agent. For example, when using Amberlite® XAD-16 as the adsorbent, the adsorbent beads may partially dry prior to use during handling, sterilization, and storage. When the water content of these adsorbents drops below a critical level, a rapid loss in adsorption capacity occurs (probably due to "collapse" of the pores); thus, for optimum effectiveness, the pores have to be "reopened" with a wetting agent prior to use.

Stabilizing agents are effective in maintaining adsorption capacity near its maximum when certain adsorbent resins are subjected to drying conditions. It is believed that the use of stabilizing agents serves to prevent the adsorbent pores from collapsing.

An acceptable stabilizing agent should be soluble in water and ethanol, nonvolatile relative to ethanol and water, and safe for transfusion in small amounts. Glycerol and low molecular weight polyethylene glycol (*e.g.*, PEG-200 and PEG-400) are examples of stabilizing agents possessing these characteristics. Glycerol has a positive hemocompatibility history. It is frequently added to blood as a cryo-preservative agent in the frozen storage of red blood cell preparations.

*See, e.g.*, Chaplin *et al.*, Transfusion 26:341-45 (1986); Valeri *et al.*, Am. J. Vet. Res. 42(9)1590-94 (1981). Solutions containing up to 1% glycerol are routinely transfused, and glycerol solutions are commercially available (*e.g.*, Glyerolite 57 Solution, Fenwal Laboratories, Deerfield, IL). Adsorbent beads like Amberlite® XAD-16 may be stabilized in ethanol and glycerol.

Low molecular weight polyethylene glycols, commonly used as pharmaceutical bases, may also be used as stabilizing agents. PEGs are liquid and solid polymers of the general chemical formula $H(OCH_2CH_2)_nOH$, where *n* is greater than or equal to 4. PEG formulations are usually followed by a number that corresponds to its average molecular weight; for example, PEG-200 has a molecular weight of 200 and a molecular weight range of 190-210. PEGs are commercially available in a number of formulations (*e.g.*, Carbowax, Poly-G, and Solbase).

*Inert Matrices for Particle Immobilization*

The adsorbent particles are immobilized by an inert matrix. The inert matrix can be made of fibrous or particulate, synthetic or natural polymer. The inert matrix can be sintered polymers. The inert matrix, as with the other components of the device, preferably is biocompatible and does not substantially adversely affect the biological activity of a material upon contact.

In an embodiment using synthetic fibers, the synthetic fibers are polyester fibers (Air Quality Filtration (AQF), a division of Hoechst Celanese (Charlotte, N.C.)). Other preferred examples of synthetic fibers are polyethylene or polyamide fibers. Other exemplary synthetic fibers include polyolefin, polyvinyl alcohol and polysulfone fibers.

In a preferred embodiment, the synthetic polymer fiber includes a first polymer core with a high melting point surrounded by a sheath with a lower melting temperature. The polymer core can be a polyester(polyethylene terephthalate). The sheath can be a nylon, or a modified polyester. Fibers are commercially available from Unitika (Osaka, Japan) and Hoechst Trevira GmbH & Co. (Augsberg, Germany).

For a fibrous matrix, the most preferred embodiment uses cellulose fibers. These cellulose fibers can be derived from a variety of sources, such as jute, kozu, kraft and manila hemp. Networks of synthetic or natural polymer fibers have been used to make filters as described in U.S. Patent Nos. 4,559,145 and 5,639,376, which are herein incorporated by reference.

A sintered matrix is also a preferred embodiment. Synthetic polymers suitable for the construction of such sintered particles are high density polyethylene, ultra high molecular weight polyethylene, polypropylene, polyvinyl fluoride, polytetrafluoroethylene, nylon 6. More preferably the sintered particles are polyolefins, such as polyethylene.

Polymeric fibers such as those described above may be adsorbent resins without the attachment of adsorbent particles. Such fibers may be formed into a fiber network or may be immobilized on a fiber network of a less adsorbent fiber. Such fibers are contemplated by the present invention; such fibers preferably contain a large, porous, adsorptive surface area or other adsorptive means to facilitate reduction in the concentration of low molecular weight compounds.

*Immobilization of Particles*

In one embodiment, the adsorbent particles are immobilized by an inert matrix to produce an adsorption medium for reducing the concentration of small organic compounds in a material. The inert matrix can be a three dimensional network including a synthetic or natural polymer fiber network with adsorbent particles immobilized therein.

Preferably, the adsorption medium comprises small porous adsorbent particles with highly porous structures and very high internal surface areas, as described above, immobilized by the inert matrix. Preferably, when a biological material is brought into contact with the adsorption medium, the adsorption medium does not substantially adversely affect the biological activity or other properties of the material.

Technology for immobilization of adsorbent beads on a fiber network to construct air filters has been described in U.S. Patent No. 5,486,410 and U.S. Patent No. 5,605,746, incorporated by reference herein. As depicted in Figure 1,

the polymer fibers 600 of the fiber network consist of a polymer core 602 (*e.g.*, polyethylene terephthalates (PET)) with a high melting point surrounded by a polymer sheath 604 (*e.g.*, nylon) with a relatively low melting temperature. *See* U.S. Patent No. 5,190,657 to Heagle *et al.*, hereby incorporated by reference. The fiberized resin is prepared by first evenly distributing the adsorbent beads in the fiber network. Next, the network is rapidly heated (*e.g.*, 180°C x 1 min.) causing the polymer sheath of the fibers 600 to melt and bond to the adsorbent beads 606 and other fibers, forming a cross-linked fiber network, represented in Figure 2. As depicted in Figure 3 and Figure 4 (not to scale), generally speaking, the fiber networks contain three layers; two outer layers 607 that are densely packed with fibers 600 and a less dense inner layer 609 that contains the adsorbent beads 606 and fewer fibers 600. In a preferred embodiment, the edges of the fiberized resin may be sealed with polyurethane or other polymers. Alternatively, as depicted in Figure3 and Figure 4, heat seals 608 may be made in the resulting fiberized resin at predetermined intervals; for example, heat seals can be made in the fiberized resin in a pattern of squares. Thereafter, the fiberized resin can be cut through the heat seals to form samples of resin containing a desired mass (*e.g.*, preferably less than 5.0 g and more preferably less than 3.0 g) of adsorbent beads and of a size suitable for placement within a blood product container. The heat seals serve to prevent the cut fiberized resin from fraying and help to immobilize the adsorbent beads. However, the use of such heat seals is not required in order to practice the present invention. In an alternative embodiment, depicted in Figure 4, the adsorbent beads 606 are not secured to the fibers themselves, but rather are immobilized between the denser outer layers 607 of fibers and with the heat seals 608; this embodiment may also result in samples of fiberized media containing a defined amount of adsorbent after being cut through the heat seals.

The present invention also contemplates the use of an adhesive (*e.g.*, a bonding agent) to secure the adsorbent resin to the fibers. Moreover, while it is preferable that the adsorbent beads be chemically attached to the fiber network, the beads may also be physically trapped within the fiber network; this might be

accomplished, for example, by surrounding the beads with enough fibers so as to hold the beads in position.

Other ways that the adsorbent particles may be immobilized in a fiber network are also contemplated. The particles can be immobilized using a dry-laid process, as described in U.S. Pat. Nos. 5,605,746 and 5,486,410 (AQF patents), which are herein incorporated by reference. The particles can be immobilized using a wet-laid process, as described in U.S. Pat. Nos. 4,559,145 and 4,309,247, which are herein incorporated by reference. The particles can be immobilized using a melt-blown process, as described in U.S. Pat. No. 5,616,254, which is herein incorporated by reference. Where a wet-laid process is used to construct a matrix from natural polymer fibers, the inert matrix preferably includes a binding agent to bond the adsorbent particles to the fibers. Nonlimiting examples of binding agents include melamine, polyamines and polyamides. The matrix typically contains 1% or less of such binding agents.

Where the inert matrix is constructed from particles of synthetic polymers which are sintered with adsorbent particles, it is important that the adsorbent particle have a higher melting temperature than the matrix.

In a preferred embodiment, the adsorbent particles are immobilized in a fiber matrix that is formed by thermal bonding of a biocomponent fiber network. An alternative embodiment involves immobilizing adsorbent particles in non-biocomponent fibers and using a wet strength resin system, adhesives or additional fusible fibers to form bonds between the fibers and adsorbent particles. Nonlimiting examples of useful fibers include polyester, nylon and polyolefin. (Suppliers of fibers for the nonwovens industry have been listed in "A Guide to Fibers for Nonwovens," *Nonwovens Industry*, June 1998, 66-87.) Examples of wet strength resin systems include melamine/formaldehyde, epichlorohydrin-based resins, polyamines and polyamides. The use of heat fusible fibers for immobilizing particles in fiber matrices has been disclosed. *See, e.g.*, U.S. Pat. No. 4,160,059.

Preferably, the resulting adsorption medium comprises known amounts of adsorbent per area. The adsorbent per area is from about 300 g/m$^2$ to about 1100

$g/m^2$, preferably from about 500 $g/m^2$ to about 700 $g/m^2$. Thus, the appropriate amount of adsorbent contemplated for a specific purpose can be measured simply by cutting a predetermined area of the fiberized resin (*i.e.*, there is no weighing of the fiberized resin).

The adsorption medium preferably is biocompatible (*i.e.*, not producing a toxic, injurious, or immunologic response); has a minimal impact on the properties of the material such as biological composition (*e.g.*, plasma); and is not associated with toxic extractables. The immobilized adsorbent particles of the adsorption medium preferably have high mechanical stability (*i.e.*, no fine particle generation). The adsorption medium contains about 20–70% adsorbent by weight, preferably 30–50% by weight. Preferably the adsorption medium contains about 30% by weight of the adsorbent particle where a fibrous matrix is used. Where a sintered particulate matrix is used with a ground polymeric adsorbent particle, the adsorption medium preferably contains about 50% by weight of the adsorbent particle.

*Coating the Adsorbent Particles*

The surface hemocompatibility of the particles, matrices or adsorption medium can be improved by coating their surfaces with a hydrophilic polymer. Exemplary hydrophilic polymers include poly(2-hydroxyethyl methacrylate) (pHEMA), which may be obtained from, *e.g.*, Scientific Polymer Products, Inc. (Ontario, NY) and cellulose-based polymers, *e.g.*, ethyl cellulose, which may be obtained from Dow Chemical (Midland, MI). *See, e.g.*, Andrade *et al.*, *Trans. Amer. Soc. Artif. Int. Organs* XVII:222-28 (1971). Other examples of coatings include polyethylene glycol and polyethylene oxide, also available from Scientific Polymer Products, Inc.. The polymer coating can increase hemocompatibility and reduce the risk of small particle generation due to mechanical breakdown.

The adsorbent surface may also be modified with immobilized heparin. In addition, strong anion exchange polystyrene divinylbenzene adsorbents may be modified via heparin adsorption. Heparin, a polyanion, will adsorb very strongly to the surfaces of adsorbents which have strong anion exchange characteristics. A

variety of quaternary amine-modified polystyrene divinyl benzene adsorbents are commercially available.

The coating can be applied in a number of different methods, including radio frequency glow discharge polymerization, as described in U.S. Patent number 5,455,040, which is hereby incorporated by reference and the Wurster coating process (performed by International Processing Corp. (Winchester, KY).

In one embodiment, the Wurster coating process can be applied by suspending the adsorbent particles (generally via air pressure) in a chamber such that the hydrophilic polymer, such as pHEMA, can be sprayed evenly onto all surfaces of the adsorbent particle. As illustrated in Example 2, Dowex® XUS-43493 sprayed evenly with pHEMA demonstrated an increase in platelet yield as well as a dramatic effect on platelet shape change with increasing amounts of coating. It was found that the Wurster coating process selectively coated the outside surface of the adsorbent surface, leaving the inside porous surface nearly unaffected.

In a preferred embodiment, the coating can be applied by soaking the immobilized adsorption medium in the hydrophilic polymer (see Example 2). This process is simpler and less expensive than spraying the adsorbent particles with the hydrophilic polymer.

The process is not limited to a process that applies the coating of the adsorption medium at any particular time. For example, in one embodiment, the pHEMA coating is applied after production of the adsorption medium, but prior to heat sealing the adsorption medium. In another embodiment, the adsorption medium is first heat sealed, and then the pHEMA coating is applied. In addition to coating the adsorption medium, the rinsing process associated with pHEMA application serves to remove loose particles and fibers.

As the amount of coating is increased, it becomes more difficult for some compounds to cross the coating to reach the particle surface, resulting in a decrease in adsorption kinetics. Thus, as the amount of coating is increased, an increased mass of adsorbent must generally be used to achieve the same removal kinetics as coated adsorbent. In one embodiment, the optimum level of pHEMA

coating is the minimum coating at which a protective effect on plasma function is observed.

The coatings may be sensitive to sterilization. For example, gamma sterilization may result in cross-linking and/or scission of the coating. Therefore, the type (E-beam vs. gamma irradiation) and dose of sterilization may influence the properties of the coated adsorbent. Generally, E-beam sterilization is preferred.

*Devices*

Devices are provided for reduction of concentration of compounds from biological compositions. The compounds have molecular weights ranging from about 100 g/mol to about 30,000 g/mol. The device is a flow device. An example of a flow device is shown in Figure 12. Flow devices are known in the literature and are described, for example, in PCT publication WO 96/40857, incorporated by reference herein.

Flow devices permit reduction of concentration of low molecular weight compounds from materials such as blood products by perfusing the blood product through the flow device.

The adsorption medium of the flow device is preferably about 3 to 30 mm thick to promote an even flow of biological fluid without a substantial pressure drop. Preferably the medium is about 3 to 15 mm thick. More preferably, the medium is about 5 to 8 mm thick.

In one embodiment, the device is a disk configuration flow device. A disk configuration flow device is shown in reference to Figure 14, which is an exploded view of the device embodiment. A biological composition to be treated with the device flows through the tubing connector for housing inlet (1). The biological composition then flows through the IAD (Immobilized Adsorption Device) housing inlet (2) and into IAD media (3), which reduces the concentration of a low molecular weight compound in the biological composition. The biological composition then may flow through a pre-filter (4), which is optional. It then flows through a membrane (5) that removes particulate matter

from the composition. Finally the treated biological composition exits the device through the IAD housing outlet (6).

In another embodiment, the device is a drip chamber configuration (Porex IAD). A drip chamber configuration flow device is shown in reference to Figure 15, which is an exploded view. The biological composition to be treated with the device flows through the IAD (Immobilized Adsorption Device) housing inlet (60). The biological composition then flows into the IAD housing (drip chamber) (50) and further into the IAD media (40), which reduces the concentration of a low molecular weight compound in the biological composition. The biological composition then may flow through a pre-filter (30), which is optional. It then flows through a membrane (20) that removes particulate matter from the composition. Finally, the treated biological composition exits the device through the IAD housing outlet (10).

*Preferred Embodiments for Biological Compositions*

In some embodiments, the present invention provides devices for reducing the concentration of compounds in a biological composition. The devices include an adsorption medium and are of a flow configuration. The compounds have a molecular weight ranging from about 100 g/mol to about 30,000 g/mol. The biological activity of the biological composition is substantially maintained after contact with such devices.

Biological response modifiers (e.g., activated complement) like the anaphalatoxin C3a and the terminal membrane attack complex SC5b-9 have been shown to be produced by the processing, (e.g., leukofiltration, pheresis, recovery of shed blood, etc.) and storage of whole blood and its components. These biological response modifiers have been implicated in adverse events in surgery and transfusion.

In some embodiments, the device of the present invention reduces the concentration of activated complement in biological compositions. The concentration of activated complement in the composition is reduced when it is treated with the device, as opposed to a composition that has not been treated with the device. In one embodiment, exposure to the device results in at least about a

30% reduction in C3a complement fragment and SC5b-9 terminal component over control. In another embodiment, exposure to the device results in at least about a 50% reduction in C3a complement fragment and SC5b-9 terminal component over control. In another embodiment, exposure to the device results in at least about a 90% reduction in C3a complement fragment over control.

In one embodiment, the invention provides a device for reducing the concentration of compounds in a biological composition comprising plasma. Treatment of the biological composition comprising plasma with the device substantially maintains the biological activity of the plasma. The adsorption medium comprises adsorbent particles immobilized by an inert matrix. Preferred particles are highly porous and have a surface area greater than about 750 $m^2/g$.

Particularly preferred particles are Norit A Supra, which is available from Norit Americas, Inc. (Atlanta, GA). Norit A Supra is a USP-grade activated carbon that is formed by steam activation of coconut shells. This activated carbon has a very high total surface area (2000 $m^2/g$) and is very microporous in nature.

Additionally, the particles may be selected from any of the following particles wherein the particles preferably possess a size range of about 1 μm to about 200 μm in diameter, either by grinding direct synthesis, or some other means, and are activated carbons, such as Picactif Medicinal (Pica USA, Columbus, OH), synthetic carbonaceous adsorbents, such as Ambersorb 572 (Rohm and Haas, Philadelphia, PA), hydrophobic resins, such as Amberlite adsorbents (*e.g.*, Amberlite® XAD-2, XAD-4, and XAD-16), available from Rohm and Haas (Philadelphia, PA); Amberchrom® adsorbents available from Toso Haas (TosoHass, Montgomeryville, PA); Diaion®//Sepabeads® Adsorbents (*e.g.*, Diaion® HP20), available from Mitsubishi Chemical America, Inc. (White Plains, NY); Hypersol-Macronet® Sorbent Resins (*e.g.*, Hypersol-Macronet® Sorbent Resins MN-150 and MN-400) available from Purolite (Bala Cynwyd, PA) and Dowex® Adsorbents (*e.g.*, Dowex® XUS-40323, XUS-43493, and XUS-40285), available from Dow Chemical Company (Midland, MI).

The inert matrix may be composed of synthetic or natural polymeric fibers or particles. In preferred embodiments the matrix is fibrous cellulose or sintered particles of ultra high molecular weight polyethylene.

Exemplary compounds that are reduced or controlled by the devices, materials and methods of the present invention are psoralens, psoralen derivatives, isopsoralens, psoralen photoproducts, methylene blue, phenothiazine, acridine, plastic extractables, biological response modifiers, quenchers and polyamine derivatives.

Where the device is used for compositions comprising plasma, the device maintains adequate levels of clotting activity. Measures of clotting activity include prothrombin time (PT), activated partial thromboplastin time (aPTT), and functional measures of clotting factors I, II, V, VII, VIII, IX, X, XI, and XII. An adequate functional measure of clotting factor activity is greater than about 80% of the level prior to passing through the device, or in the case of clotting times, one that remains in the normal range established for each laboratory that does this type of testing. Preferred measures of clotting activity include PT and aPTT, as they are measures of the overall ability of the plasma to clot, and factors I, II, V, VII, X, XI, and XII, as these factors are not commonly replaced by recombinant proteins. It is preferred to retain more than about 90% of the clotting activity of these factors relative to the level prior to passing through the device and have changes in PT and aPTT of less than about 1.5 seconds.

In one embodiment the device may comprise an adsorption medium and a housing. The housing should promote even flow of the plasma to promote good media utilization and as it primes, allow the plasma to push air ahead of it, thereby eliminating bubbles that would reduce the contact area between the plasma and the adsorption media, thereby reducing the media's utilization. The housing can be flat or have substantial depth to accommodate adsorption media or particle retention media that is not flat, for example cylindrically shaped adsorption media. In a preferred embodiment, the housing is flat. The housing can have inlets and outlets in various orientations, for example inlet top/outlet top or inlet

bottom/outlet bottom. In a preferred embodiment, the outlet is at the bottom to promote good drainage and the inlet is at the top to promote media utilization.

In another embodiment, the device comprising an adsorption medium and a housing may also include a particle retention medium. In a preferred embodiment the device includes a particle retention medium downstream of the adsorption medium to retain particles that are shed from the adsorption medium while maintaining a high fluid flow rate and high recovery of proteins. The particle retention medium can be a membrane, a dry or wet laid matrix of fibers, a sintered polymer matrix, a woven material, a nonwoven material (polyester nonwoven), or a combination thereof.

The device housing holds the particle retention medium in an approximately parallel orientation downstream of the adsorption medium. (U.S. Patent No. 5,660,731, which is herein incorporated by reference, discloses examples of filter housings.) The housing can be constructed from any suitably rigid, impervious, material that does not substantially adversely affect the biological activity of a fluid. Preferably the housing is constructed from a synthetic polymer. Nonlimiting examples of such polymers include polyacrylic, polyethylene, polypropylene, polystyrene and polycarbonate plastics.

The adsorption medium of the device containing particles immobilized by an inert matrix should be between 3 and 30 mm thick to promote an even flow of biological fluid without a substantial pressure drop. Preferably the medium should be between 3 and 15 mm thick. More preferably, the medium should be between 5 and 8 mm thick.

For the device, gravity flow is preferred. More preferably, the device is a gravity flow device that is constructed to permit flow rates of 0.1-10 $mL/cm^2/min$ with differential pressures of 12-72 inches water. More preferably the device permits flowrates of 0.2-5 $mL/cm^2/min$ with differential pressures of 24-48 inches of water.

*Applications*

The present invention contemplates reducing the concentration of low molecular weight compounds in biological compositions. The compounds have a

molecular weight ranging from about 100 g/mol to about 30,000 g/mol. Such compounds include, for example, pathogen-inactivating agents such as photoactivation products, aminoacridines, organic dyes and phenothiazines. Exemplary pathogen inactivating agents include furocoumarins, such as psoralens and acridines. Following treatment of a blood product with a pathogen inactivating compound as described for example in U.S. Patent Numbers 5,459,030 and 5,559,250, incorporated by reference herein, the concentration of pathogen inactivating compounds in the blood product can be reduced by contacting the treated blood product with a device of the invention.

In one embodiment the present invention contemplates a method of inactivating pathogens in solution, wherein the method comprises: a) providing, in any order: i) a cyclic compound, ii) a solution suspected of being contaminated with said pathogens, and iii) fiberized resin; b) treating said solution with said cyclic compound so as to create a treated solution product wherein said pathogens are inactivated; and c) contacting said treated solution product with said fiberized resin, and further comprising a device for reducing the concentration of small organic compounds in a blood product while substantially maintaining a desired biological activity of the blood product, the device comprising highly porous adsorbent particles, wherein the adsorbent particles are immobilized by an inert matrix.

In addition to the pathogen inactivating compound, reactive degradation products thereof can be reduced from the material such as a blood product, for example prior to transfusion.

The materials and devices disclosed herein can be used in apheresis methods. Whole blood can be separated into two or more specific components (*e.g.*, red blood cells, plasma and platelets). The term "apheresis" refers broadly to procedures in which blood is removed from a donor and separated into various components, the component(s) of interest being collected and retained and the other components being returned to the donor. The donor receives replacement fluids during the reinfusion process to help compensate for the volume and

pressure loss caused by component removal. Apherersis systems are described in PCT publication WO96/40857, hereby incorporated by reference.

*Low Molecular Weight Compounds*

A device of the present invention reduces the concentration of low molecular weight compounds in a biological composition. The term "low molecular weight compound" refers to an organic or biological molecule having a molecular weight ranging from about 100 g/mol to about 30,000 g/mol. Low molecular weight compounds include, without limitation, the following compounds: small organic compounds such as psoralens, acridines or dyes; quenchers, such as glutathione; plastic extractables, such as plasticizers; biological modifiers, such as activated complement, that possess a molecular weight between about 100 g/mol and about 30,000 g/mol; and, polyamine derivatives.

*Small Organic Compounds*

A diverse set of small organic compounds can be adsorbed by the device of the present invention. The molecules can be cyclic or acyclic. In one embodiment the compounds are preferably, cyclic compounds such as psoralens, acridines or dyes. In another embodiment the compounds are thiols.

Nonlimiting examples of cyclic compounds include actinomycins, anthracyclinones, mitomyacin, anthramycin, and organic dyes and photoreactive compounds such as benzodipyrones, fluorenes, fluorenones, furocoumarins, porphyrins, protoporphyrins, purpurins, phthalocyanines, hypericin, Monostral Fast Blue, Norphillin A, phenanthridines, phenazathionium salts, phenazines, phenothiazines, phenylazides, quinolines and thiaxanthenones. Preferably the compounds are furocoumarins or organic dyes. More preferably the compounds are furocoumarins.

Nonlimiting examples of furocoumarins, include psoralens and psoralen derivatives. Specifically contemplated are 4'-aminomethyl-4,5',8-trimethylpsoralen, 8-methoxypsoralen, halogenated psoralens, isopsoralens and psoralens linked to quaternary amines, sugars, or other nucleic acid binding groups. Also contemplated are the following psoralens: 5'-bromomethyl-4,4',8-

trimethylpsoralen, 4'-bromomethyl-4,5',8-trimethylpsoralen, 4'-(4-amino-2-aza)butyl-4,5',8-trimethylpsoralen, 4'-(4-amino-2-oxa)butyl-4,5',8-trimethylpsoralen, 4'-(2-aminoethyl)-4,5',8-trimethylpsoralen, 4'-(5-amino-2-oxa)pentyl-4,5',8-trimethylpsoralen, 4'-(5-amino-2-aza)pentyl-4,5',8-trimethylpsoralen, 4'-(6-amino-2-aza)hexyl-4,5',8-trimethylpsoralen, 4'-(7-amino-2,5-oxa)heptyl-4,5',8-trimethylpsoralen, 4'-(12-amino-8-aza-2,5-dioxa)dodecyl-4,5',8-trimethylpsoralen, 4'-(13-amino-2-aza-6,11-dioxa)tridecyl-4,5',8-trimethylpsoralen, 4'-(7-amino-2-aza)heptyl-4,5',8-trimethylpsoralen, 4'-(7-amino-2-aza-5-oxa)heptyl-4,5',8-trimethylpsoralen, 4'-(9-amino-2,6-diaza)nonyl-4,5',8-trimethylpsoralen, 4'-(8-amino-5-aza-2-oxa)octyl-4,5',8-trimethylpsoralen, 4'-(9-amino-5-aza-2-oxa)nonyl-4,5',8-trimethylpsoralen, 4'-(14-amino-2,6,11-triaza)tetradecyl-4,5',8-trimethylpsoralen, 5'-(4-amino-2-aza)butyl-4,4',8-trimethylpsoralen, 5'-(6-amino-2-aza)hexyl-4,4',8-trimethylpsoralen and 5'-(4-amino-2-oxa)butyl-4,4',8-trimethylpsoralen. Preferably, the psoralen is 4'-(4-amino-2-oxa)butyl-4,5',8-trimethylpsoralen.

*Acridines*

Nonlimiting examples of acridines include acridine orange, acriflavine, quinacrine, N1, N1-bis (2-hydroxyethyl)-N4-(6-chloro-2-methoxy-9-acridinyl)-1,4-pentanediamine, 9-(3-hydroxypropyl)aminoacridine, N-(9-acridinyl)glycine, S-(9-acridinyl)-glutathione. In a preferred embodiment the acridine is N-(9-acridinyl)-β-alanine, alternatively, named 5-[(β-carboxyethyl)amino]acridine.

*Dyes*

Nonlimiting examples of dyes include phenothiazines such as methylene blue, neutral red, toluidine blue, crystal violet and azure A, phenothiazones such as methylene violet Bernthsen, phthalocyanines such as aluminum 1,8,15,22-tetraphenoxy-29H,31H-phthalocyanine chloride and silica analogues, and hypericin. Preferably, the dye is methylene blue or toluidine blue. More preferably, the dye is methylene blue.

The term "thiazine dyes" includes dyes that contain a sulfur atom in one or more rings. The most common thiazine dye is methylene blue [3,7-Bis(dimethylamino)-phenothiazin-5-ium chloride). Other thiazine dyes include,

but are not limited to, azure A, azure C and thionine, as described *e.g.* in U.S. Patent No. 5,571,666 to Schinazi.

The term "xanthene dyes" refers to dyes that are derivatives of the compound xanthene. The xanthene dyes may be placed into one of three major categories: i) fluorenes or amino xanthenes, ii) the rhodols or aminohydroxyxanthenes, and iii) the fluorones or hydroxyxantheses. Examples of xanthene dyes contemplated for use with the present invention include rose bengal and eosin Y; these dyes may be commercially obtained from a number of sources (*e.g.*, Sigma Chemical Co., St. Louis, MI), and as described *e.g.* in U.S. Patent No. 5,571,666 to Schinazi, hereby incorporated by reference.

*Quenchers*

The concentration of a variety of compounds may be reduced. Other exemplary compounds include quenching compounds. Methods for quenching undesired side reactions of pathogen inactivating compounds that include a functional group which is, or which is capable of forming, an electrophilic group, are described in the co-filed U.S. Patent Application, "Methods for Quenching Pathogen Inactivators in Biological Systems", Docket Number 282173000600, filed January 6, 1998, the disclosure of which is incorporated herein. In this method, a material, such as a blood product, is treated with the pathogen inactivating compound and a quencher, wherein the quencher comprises a nucleophilic functional group that is capable of covalently reacting with the electrophilic group. In one embodiment, the pathogen inactivating compound includes a nucleic acid binding ligand and a functional group, such as a mustard group, which is capable of reacting *in situ* to form the electrophilic group. Examples of quenchers include, but are not limited to, compounds including nucleophilic groups. Exemplary nucleophilic groups include thiol, thioacid, dithoic acid, thiocarbamate, dithiocarbamate, amine, phosphate, and thiophosphate groups. The quencher may be, or contain, a nitrogen heterocycle such as pyridine. The quencher can be a phosphate containing compound such as glucose-6-phosphate. The quencher also can be a thiol containing compound, including, but not limited to, glutathione, cysteine, N-acetylcysteine,

mercaptoethanol, dimercaprol, mercaptan, mercaptoethanesulfonic acid and salts thereof, *e.g.*, MESNA, homocysteine, aminoethane thiol, dimethylaminoethane thiol, dithiothreitol, and other thiol containing compounds. Exemplary aromatic thiol compounds include 2-mercaptobenzimidazolesulfonic acid, 2-mercapto-nicotinic acid, napthalenethiol, quinoline thiol, 4-nitro-thiophenol, and thiophenol. Other quenchers include nitrobenzylpyridine and inorganic nucleophiles such as selenide salts or organoselenides, thiosulfate, sulfite, sulfide, thiophosphate, pyrophosphate, hydrosulfide, and dithionitrite. The quencher can be a peptide compound containing a nucleophilic group. For example, the quencher may be a cysteine containing compound, for example, a dipeptide, such as GlyCys, or a tripeptide, such as glutathione.

Compounds that may be removed by the device of the present invention may include thiols such as methyl thioglycolate, thiolactic acid, thiophenol, 2-mercaptopyridine, 3-mercapto-2-butanol, 2-mercaptobenzothiazole, thiosalicylic acid and thioctic acid.

*Plastic Extractables*

The concentration of a group of low molecular weight compounds that are extractables from plastic storage containers and tubing used to handle biological compositions may also be reduced in a biological composition using a device of the present invention. Examples of extractables include, but are not limited to, plasticizers, residual monomer, low molecular weight oligomers, antioxidants and lubricants. *See, e.g.*, R. Carmen, *Transfusion Medicine Reviews* 7(1):1-10 (1993). The sterilization of plastic components by steam, gamma irradiation or electron beam can produce oxidative reactions and/or polymer scission, resulting in the formation of additional extractable species.

Plasticizers are commonly used to enhance properties of plastics such as processability and gas permeability. The most common plasticizer found in blood storage containers is di(2-ethylhexyl) phthalate (DEHP), which is used in PVC formulations. DEHP has been identified as a potential carcinogen. Alternative plasticizers have been developed, including, without limitation, the following

compounds: tri (2-ethylhexyl) trimellitate (TEHTM), acetyl-tri-n-hexyl citrate (ATHC), butyryl-tri-n-hexyl-citrate (BTHC), and di-n-decyl phthalate.

A device of the present invention may be used to reduce or control the concentration of plastic extractables in a biological composition in a variety of settings. Such settings include, but are not limited to, the following: blood treatment; blood storage; and, extracorporeal applications such as hemodialysis and extracorporeal membrane oxygenation.

*Biological Response Modifiers (BRMs)*

The concentration of a group of low molecular weight compounds broadly referred to as biological response modifiers (BRMs) may also be reduced in a biological composition using a device of the present invention. BRMs are defined as "a wide spectrum of molecules that alter the immune response." *Illustrated Dictionary of Immunology*, J.M. Cruse and R.E. Lewis. General groups of BRMs include, without limitation, the following types of compounds: small molecules such as histamine and serotonin; lipids such as thromboxanes, prostaglandins, leukotrienes and arachidonic acid; small peptides such as bradykinin; larger polypeptides that contain further groups, including activated complement fragments (C3a, C5a); cytokines such as IL-1, IL-6 and IL-8; and chemokines such as RANTES and MIP.

The accumulation of BRMs in a blood product during storage can adversely affect the desired biological activity of a biological composition. Complement activation, for example, has been demonstrated to occur during storage of platelets under standard blood bank conditions. Complement activation has been associated with a loss of platelet function and viability termed "platelet storage lesion." *See, e.g.*, V.D. Mietic and O. Popovic, *Transfusion* 33(2):150-154 (1993). The accumulation of BRMs in a stored blood products can also, for example, adversely affect a patient that receives the blood product: the accumulation of BRMs in platelet concentrates during storage has been associated with non-hemolytic febrile transfusion reactions in patients receiving platelets. *See, e.g.*, N.M. Heddle, *Current Opinions in Hematology* 2(6):478-483 (1995).

*Polyamine Derivatives*

The concentration of a group of low molecular weight compounds known as polyamine derivatives may also, for example, be reduced in a biological composition using a device of the present invention. Polyamine derivatives are compounds that contain multiple nitrogen atoms in a carbon backbone.

*Polyethylene Glycols*

Other exemplary compounds include activated polyethylene glycols (aPEG), which may be used for the modification of the surface of cells or materials in order to provide immunomasking properties or pacification toward protein binding, respectively. The device may be used for the reduction of either the excess activated polyethylene glycol or the unreactive derivative of the PEG resulting from the reaction of the activated PEG with water or small nucleophiles such as phosphate, phosphate esters or thiols, such as glutathione. Other compounds that may be removed include impurities in the activated PEG preparation, which may affect the function of the blood products or make them unsuitable for transfusion (eg. toxic compounds). Finally, small molecules (leaving groups) such as N-Hydroxy succinimide which are released during the reaction of the aPEG with cell surface nucleophiles may also be reduced.

Examples of compounds that may be removed by the device of the present invention include linear or branched polyethylene glycols attached to activating moeities which may include cyanuryl chloride, succinimidyl esters, oxycarbonyl imidazole derivatives, nitrophenyl carbonate derivatives, glycidyl ether derivatives, and aldehydes.

## EXAMPLES

The following examples serve to illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); Kg (kilograms); L (liters); mL (milliliters);

μL(microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); min. (minutes); s and sec. (seconds); J (Joules, also watt second); °C (degrees Centigrade); TLC (Thin Layer Chromatography); HPLC (high pressure liquid chromatography); pHEMA and p(HEMA) (poly[2-hydroxyethyl methacrylate]); PC(s) (platelet concentrate(s)); PT (prothrombin time); aPTT (activated partial thromboplastin time); TT (thrombin time); HSR (hypotonic shock response); FDA (United States Food and Drug Administration); GMP (good manufacturing practices); DMF (Drug Masterfiles); SPE (Solid Phase Extraction); Aldrich (Milwaukee, WI); Asahi (Asahi Medical Co., Ltd., Tokyo, Japan); Baker (J.T. Baker, Inc., Phillipsburg, NJ); Barnstead (Barnstead/Thermolyne Corp., Dubuque, IA); Becton Dickinson (Becton Dickinson Microbiology Systems; Cockeysville, MD); Bio-Rad (Bio-Rad Laboratories, Hercules, CA); Cerus (Cerus Corporation; Concord, CA); Chrono-Log (Chrono-Log Corp.; Havertown, PA); Ciba-Corning (Ciba-Corning Diagnostics Corp.; Oberlin, OH); Consolidated Plastics (Consolidated Plastics Co., Twinsburg, OH); Dow (Dow Chemical Co.; Midland, MI); Eppendorf (Eppendorf North America Inc., Madison, WI); Gelman (Gelman Sciences, Ann Arbor, MI); Grace Davison (W.R. Grace & Co., Baltimore, MD); Helmer (Helmer Labs, Noblesville, IN); Hoechst Celanese (Hoechst Celanese Corp., Charlotte, NC); International Processing Corp. (Winchester, KY); Millipore (Milford, MA); NIS (Nicolet, a Thermo Spectra Co., San Diego, CA); Poretics (Livermore, CA); Purolite (Bala Cynwyd, PA); Quidel (San Diego, CA); Rohm and Haas (Chauny, France); Saati (Stamford, CT); Scientific Polymer Products (Ontario, NY); Sigma (Sigma Chemical Company, St. Louis, MO); Spectrum (Spectrum Chemical Mfg. Corp., Gardenia, CA); Sterigenics (Corona, CA); Tetko, Inc. (Depew, NY); TosoHaas (TosoHass, Montgomeryville, PA); Wallac (Wallac Inc., Gaithersburg, MD); West Vaco (Luke, W.Va.); YMC (YMC Inc., Wilmington, NC); DVB (divinyl benzene); LAL (Limulus Amoebocyte Lystate); USP (United States Pharmacopeia); EAA (ethyl-acetoacetate); EtOH (ethanol); HOAc (acetic acid); W (watts); mW (milliwatts); NMR (Nuclear Magnetic Resonance; spectra obtained at room temperature on a Varian Gemini 200 MHz Fourier Transform

Spectrometer); $ft^3$/min (cubic feet per minute); m.p. (melting point); g/min and gpm (gallons per minute); UV (ultraviolet light); THF (tetrahydrofuran); DMEM (Dulbecco's Modified Eagles Medium); FBS (fetal bovine serum); LB (Luria Broth); EDTA (ethelene diamine tetracidic acid); Phorbol Myristate Acetate (PMA); phosphate buffered saline (PBS); AAMI (Association for the Advancement of Medical Instruments); ISO (International Standards Organization); EU (endotoxin units); LVI (large volume injectables); GC (gas chromatography); M (mega-); kGy (1000 Gray = 0.1 MRad); MΩ (Mohm); PAS III (platelet additive solution III); $dH_2O$ (distilled water); IAD (immobilization adsorption device); SCD (sterile connection [connect] device).

One of the examples below refers to HEPES buffer. This buffer contains 8.0 g of 137 mM NaCl, 0.2 g of 2.7 mM KCl, 0.203 g of 1 mM $MgCl_2(6H_20)$, 1.0 g of 5.6 mM glucose, 1.0 g of 1 mg/ml Bovine Serum Albumin (BSA) (available from Sigma, St. Louis, MO), and 4.8 g of 20 mM HEPES (available from Sigma, St. Louis, MO).

## EXAMPLE 1

### Preparation of Fiberized Resin

#### Preparation Of Fiberized Resin And Adsorbent Beads

Immobilized adsorbent media containing Amberlite® XAD-16 in a cleaned and hydrated state (Rohm and Haas) was obtained from AQF. The fibers of Hoechst Celanese's fiber network consisted of a polyethylene terephthalate core and a nylon sheath, the sheath having a lower melting temperature than the core. The fiberized resin was prepared by first evenly distributing the adsorbent beads in the fiber network. Next, the fiber network was rapidly heated causing the polymer sheath of the fibers to melt and bond to the adsorbent beads and other fibers, forming a cross-linked fiber network. The fiberized resin formed contained the Amberlite® XAD-16 at a loading of 130 $g/m^2$ (*i.e.*, each square meter of fiber contained 130 g of adsorbent beads).

The fiberized resin was cut into squares (14 cm x 14 cm), and the resulting sections contained approximately 2.5 g of dry Amberlite® XAD-16. The Amberlite® XAD-16 beads were then pre-wet by soaking the fiberized resin in 30% ethanol for approximately 10 minutes. The residual ethanol was then removed by rinsing twice in saline for 10 minutes. Alternative methods of wetting the Amberlite® XAD-16 and other adsorbents are also effective and are contemplated by the present invention. It should be noted that fiberized resin containing other types of beads (*e.g.*, bridged or hypercrosslinked resins like Dowex® XUS-43493) do not require a wetting step for effective psoralen removal.

Amberlite® XAD-16 HP (High Purity) beads were also obtained directly from Rohm and Haas in a cleaned and hydrated state. No pre-wetting was required for the loose (*i.e.*, not immobilized) Amberlite® XAD-16 HP beads prior to incorporation into a mesh pouch; however, the mass of adsorbent was corrected to account for the water content of the beads (2.5 g dry = 6.8 g with 62.8% moisture). The Dowex® XUS-43493 beads were obtained from Dow, and the dry beads did not require wetting nor did the mass of the beads require correction for water. Polyester mesh pouches (7 cm x 7 cm square; 30 μm openings) were then filled with 2.5 g (dry weight) of either the loose Amberlite® XAD-16 HP or Dowex® XUS-43493 beads.

The fiberized resin and adsorbent-containing pouches were sterilized by autoclaving on "wet" cycle for 45 minutes at 121°C. Thereafter, the fiberized resin and the adsorbent-containing pouches were inserted into separate, sterile, 1-liter PL 2410 Plastic containers (Baxter). Following insertion, the PL 2410 Plastic containers were heat sealed in a laminar flow hood, using sterile scissors, hemostats, and an impulse sealer.

## EXAMPLE 2

### Preparation Of pHEMA-Coated Adsorbent Beads And Fiberized Resin

Dowex® XUS-43493 (commercially known as Optipore® L493) containing approximately 50% water by weight was obtained from Dow, and

polymerized HEMA with a viscosity average molecular weight of 300 kD was obtained from Scientific Polymer Products. Prior to coating, the adsorbent beads were dried to a water content of < 5%. A stock solution of pHEMA was prepared by dissolving the polymer in 95% denatured ethanol/5% water to achieve a pHEMA concentration of 50 mg/ml.

The coating process was performed by International Processing Corp. in a 9-inch Wurster fluidized bed coater with a charge of approximately 4 kg (dry) of adsorbent. The coating process involved a pHEMA flow rate of 60-70 g/min, an inlet temperature of 50°C, and an air flow rate of approximately 200 $ft^3$/min. Samples (50 g) of coated adsorbent were removed during the coating process so that coating levels ranging from 3-18% (w/w) pHEMA were obtained; adsorbent beads coated with 3.7%, 7.3%, and 10.9% pHEMA (w/w) were used in the studies described below.

A device containing non-immobilized dry (uncoated) Dowex® XUS-43493 (2.5 g) and pHEMA-coated Dowex® XUS-43493 (3.0 g or 5.0 g) were prepared by placing the desired mass of adsorbent into a square 30 μm polyester mesh pouch (7 cm x 7 cm). The adsorbent-filled pouches were inserted into separate sterile, 1-liter PL 2410 Plastic containers (Baxter) and heat sealed with an impulse sealer. Thereafter, the adsorbent-filled pouches containing PL-2410 Plastic containers were sterilized by either E-beam (NIS) or gamma irradiation (SteriGenics) to 2.5 MRad; as previously alluded to, E-beam sterilization is generally preferred.

Hoechst Celanese prepared fiberized resin containing Amberlite® XAD-16 according to the method described in Example 1. The fiberized resin was cut into squares (14 cm x 14 cm); the resulting sections contained approximately 2.5 g of dry Amberlite® XAD-16. The Amberlite® XAD-16 of the fiberized resin was simultaneously wet and coated with pHEMA by soaking in a solution containing 50 mg/mL pHEMA in 95% ethanol/5% distilled water. Residual ethanol was removed by rinsing twice in saline for 10 minutes. This procedure resulted in a coating of approximately 6% (w/w) pHEMA. The fiberized resin was then sterilized by autoclaving on "wet" cycle for 45 minutes at 121°C. Thereafter, the

fiberized resin was inserted into separate sterile, 1-liter PL 2410 Plastic containers (Baxter) and heat sealed in a laminar flow hood, using sterile scissors, hemostats, and an impulse sealer.

## EXAMPLE 3

### Effect Of Glycerol And Polyethylene Glycol On Adsorbent Capacity

This example examines the effect of glycerol and polyethylene glycol as stabilizing agents on adsorbent capacity and kinetics of removal of aminopsoralens from plasma. Free (*i.e.*, not fiberized) Amberlite® XAD-16 and Dowex® XUS-43493 adsorbent beads were used in the experiments of this example.

### Methodology

Amberlite® XAD-16 HP (Rohm & Haas (Philadelphia, PA)) and Dowex® XUS-43493 (Supelco, Bellefonte, PA) were dried to < 5% water in a 80°C oven. Known masses of adsorbent were soaked in ethanol solutions containing 0-50% glycerol, 50% PEG-200 or 50% PEG-400 (glycerol, PEG-200, and PEG-400 from Sigma). Following a 15 minute incubation period at room temperature, the excess solvent was removed and the samples were dried overnight in a 80°C oven; drying the adsorbent at temperatures > 120°C was avoided since changes in adsorbent properties (*e.g.*, pore melting) were previously observed at higher temperatures. After drying, adsorbent samples were weighed to determine the mass of stabilizing agent per mass of adsorbent.

Several individual studies were performed. Control samples of "non-wet" adsorbent and "optimally wet" adsorbent were included in the studies as described below. The non-wet samples of adsorbent were dried adsorbent which was not subjected to any pre-treatment, while the optimally wet samples of adsorbent were prepared by wetting the adsorbent with 30% ethanol/70% $dH_2O$. The optimally-wet adsorbent was rinsed with $dH_2O$ to remove residual ethanol. The adsorbent was prepared just prior to the adsorption study to assure that drying did not occur.

Each of the adsorption studies was performed using 100% human plasma containing 150 μM 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen spiked with $^3$H-4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen. Plasma (6.0 mL) was added to vials containing adsorbent treated with different stabilizing agents. Masses of adsorbent were corrected for glycerol or PEG content to give 0.2 g of adsorbent. The vials were placed on a rotator and agitated at room temperature. Plasma samples were removed at various times and levels of residual $^3$H-4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen were determined. Samples (200 μL) were diluted in 5.0 mL of Optiphase HiSafe Liquid Scintillation Cocktail (Wallac) and were counted on a Wallac 1409 Liquid Scintillation Counter (Wallac).

Adsorption Capacities Of Amberlite® XAD-16
And Dowex® XUS-43493 Treated With Glycerol

FIG. 7 compares the effect of pre-treatment with ethanol solutions containing various levels of glycerol on relative 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen adsorption capacity in 100% plasma for Amberlite® XAD-16 and Dowex® XUS-43493. Adsorbent samples were wet in the ethanol/glycerol solutions for 15 minutes prior to drying for 48 hours at 80°C. Single measurements of adsorption capacity were made after 4 hours of contact. Referring to FIG. 7, glycerol content shown on the x-axis is weight/volume percent of glycerol in ethanol. Adsorption capacities shown on the y-axis are percentages relative to the adsorption capacity of the optimally wet adsorbent sample. The adsorption capacity of XUS-43493 is represented by the squares, while that of XAD-16 is represented by the circles.

As the data in FIG. 7 indicate, the capacity of XAD-16 increased from about 30% in the dry sample to over 90% in the sample wet in a 20% glycerol solution. These results indicate that very low levels of glycerol are required for maintaining high adsorbent capacity after drying. Control samples that were wet in 50% ethanol/50% $dH_2O$ (no glycerol) prior to drying demonstrated adsorption capacities which were similar to untreated samples that were dried. In contrast, the XUS-43493 samples did not show any effect of glycerol on adsorption

capacity; adsorption capacity approached 100% at all levels of glycerol. While not critical to the practice of the present invention, this observation supports the hypothesis that glycerol acts to prevent the adsorbent pores from collapsing during drying; because XUS-43493 has a highly crosslinked structure, it is not subject to pore collapse upon drying.

Samples that were treated with glycerol appeared to be very stable to drying. No changes were observed in adsorption capacity for samples that were stored for 7 days in a laminar flow hood (data not shown).

Adsorption Capacities Of Amberlite® XAD-16
And Dowex® XUS-43493 Treated With Glycerol Or PEG

Additional studies were performed with the low molecular weight polyethylene glycols PEG-200 and PEG-400, low-toxicity agents that are nonvolatile and are soluble in ethanol and water. Samples of adsorbent were treated for 15 minutes in 50% solutions of PEG-400, PEG-200 or glycerol in ethanol. FIG. 8 compares the effect of the stabilizing agents on 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen adsorption capacities with dried adsorbent in 100% plasma for Amberlite® XAD-16 (bottom) and Dowex® XUS-43493 (top); the samples that were not wet are labeled "No Tx". Adsorbent capacities are reported as percentages relative to the capacity of optimally wet adsorbent.

As indicated by the data in FIG. 8 and predictable based on the its "macronet" structure, the capacity of Dowex® XUS-43493 was not affected by drying ("No Tx" sample). Conversely, the Amberlite® XAD-16 had approximately 35% of the maximum capacity when dried. Treating XAD-16 with glycerol, PEG-200, and PEG-400 all improved the capacity of the dried adsorbent; the adsorbent capacities with each were all greater than 90%, with glycerol>PEG-200>PEG-400. Though an understanding of the precise mechanism of action is not required to practice the present invention, differences in capacity between the glycerol and the two PEG solutions may be caused by decreasing penetration of the stabilizing agent with increasing molecular weight. That is, during the 15 minute application procedure, the glycerol (MW = 92.1) may be able to penetrate the adsorbent pores more completely than either PEG-

200 (MW = 190-210) or PEG-400 (MW = 380-420), which diffuse more slowly because of their larger size.

Adsorption Kinetics Of Amberlite® XAD-16 Treated With Glycerol Or PEG

A study was also performed to determine whether filling the pores of the adsorbent with glycerol or PEG results in reduced adsorption kinetics. FIG. 9 compares adsorption of 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen over a 3-hour period from 100% plasma using Amberlite® XAD-16 wet in several different solutions. Specifically, the data in FIG. 9 represents XAD-16 i) wet prior to drying with a 50% solution of glycerol (open squares connected by solid lines), ii) wet prior to drying with a 50% solution of PEG-400 (shaded circles connected with dashed lines), iii) pre-wet, *i.e.*, just prior to initiating the study, with 50% ethanol/50% $dH_2O$ (shaded triangles connected by dashes), and iv) not subjected to any treatment (shaded squares connected by solid lines; "No Tx"). The data in FIG. 9 demonstrate that Amberlite® XAD-16 samples that were wet in 50% glycerol/50% ethanol or 50% PEG-400/50% ethanol solutions had adsorption kinetics which were very close to the sample that was optimally wet in ethanol (*i.e.*, the sample pre-wet with ethanol). The XAD-16 sample that was dried but not treated (No Tx) achieved only about 30% removal by 3 hours.

The data presented in this example indicate that treating Amberlite® XAD-16 with stabilizing agents in the form of solutions containing 50% ethanol and 50% glycerol, PEG-200, or PEG-400 can prevent loss of adsorption capacity associated with drying. The results obtained with these stabilizing agents suggest that low molecular weight wetting agents represent viable methods for enhancing adsorbent function.

EXAMPLE 4

Removal Of Methylene Blue From FFP

This example is directed at the ability of a variety of different polymeric adsorbent materials to remove methylene blue from fresh frozen plasma.

The experiments of this example evaluated "free" adsorbent resin (*i.e.*, not incorporated into device containing non-immobilized adsorbents) and fiberized

resin. The free adsorbent resins tested were Amberlite® XAD-16 HP (Rohm and Haas), MN-200 (Purolite), and Dowex® XUS-43493 (Dow Chemical Co.). The XAD-16 HP came in a hydrated state so that no pre-treatment (*i.e.*, no wetting) was necessary, and the MN-200 was also supplied in a fully hydrated state; the XUS-43493 was dry.

Fiberized resin containing XAD-16 was prepared as generally described in Example 1. Briefly, a 2 cm x 7 cm (*i.e.*, 14 $cm^2$) strip of fiberized resin containing 130 $g/m^2$ XAD-16 was first wet in 70% ethanol and then rinsed exhaustively in distilled water.

A stock solution of methylene blue (10 mM) was prepared by dissolving U.S.P. methylene blue (Spectrum) in distilled water. The stock solution of methylene blue was added to a sample of 100% plasma to give a final concentration of 10 μM. Samples of the "free" adsorbent resin (*i.e.*, XAD-16 HP, MN-200, and XUS-43493) were weighed into 50 mL polypropylene tubes for adsorption studies. The water content of each adsorbent was determined by measuring mass loss upon drying. The mass of each adsorbent was corrected for water content so that the equivalent of 0.25 g dry adsorbent was used for each.

A 30 mL sample of the 100% plasma containing 10 μM methylene blue was added to each vial. The vials were placed on a rotator at room temperature. Samples (200 μL) were removed from each vial at 15 minute intervals and assayed for residual methylene blue by HPLC. Each sample of plasma was diluted 5-fold with sample diluent (final concentration = 35% methanol, 25 mM $KH_2PO_4$, pH = 3.5). Proteins and other macromolecules were precipitated by incubating the samples at 4°C for 30 minutes. Samples were centrifuged and the supernatant was filtered (0.2 μm) and analyzed on a C-18 reversed phase column (YMC ODS-AM, 4.6 mm x 250 mm) by running a linear gradient from 65% solvent A (25 mM $KH_2PO_4$, pH = 3.5), 35% B (Methanol) to 80% B in 20 minutes. The limit of detection for the HPLC assay was approximately 0.5 μM methylene blue.

FIG. 10 compares the kinetics of adsorption of methylene blue over a 2-hour period from 100% plasma. Referring to FIG. 10, XAD-16 HP data is

represented by open diamonds connected by dashed lines, the MN-200 data is represented by shaded triangles connected by solid lines, the XUS-43493 data is represented by open circles connected by dashed lines, and the fiberized resin containing XAD-16 is represented by shaded squares connected by solid lines. As the data indicate, the XAD-16 HP and MN-200 gave the fastest adsorption kinetics, followed by XUS-43493. The slightly slower kinetics of the XUS-43493 may be a result of slower wetting, as it was used in the dry state. Finally, the fiberized resin containing XAD-16 had the slowest adsorption kinetics. This may have resulted from poor contacting between the fiberized resin and plasma during the incubation, as a portion of the 14 $cm^2$ strip of fiberized resin was not completely submersed in the plasma throughout the adsorption study, thereby reducing the effective contact area between the adsorbent and plasma.

The data indicate that non-psoralen pathogen-inactivating compounds like the phenothiazine dyes can be removed from blood products using the resins and fiberized resin contemplated for use with the present invention.

## EXAMPLE 5

This example compares the use of different types of powdered carbon as the active component in the media and demonstrates how careful choice of the active constituent is necessary to reduce the concentration of a small organic compound and preserve the fluid's biological function. The five activated carbons illustrated in Example 5 reduce the concentration of the psoralen, 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen, in plasma by approximately the same amount. Where "A Supra" is used as the adsorbent particle, however, there is substantially better retention of clotting factors relative to the other adsorbents.

4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen was added to plasma at a concentration of 150 μM, and the plasma was illuminated in 325 mL batches with 3.0 J/cm2 UVA to inactivate pathogens. The residual 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen concentration was approximately 90 μM in the resulting plasma pool. 325 mL of illuminated plasma was pumped at 20 mL/min through 5 different types of 90 mm Cuno ZetaPlus carbon pads. Plasma

clotting factor levels, clotting times, and 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen concentration were assayed before and after flow through the carbon pads.

| Cuno Grade | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | aPTT Increase (s) | I % Yield | VIII % Yield | IX % Yield |
|---|---|---|---|---|---|
| R11S | 99.3 | 4.8 | 91 | 89 | 80 |
| R12S | 99.4 | 4.9 | 93 | 89 | 48 |
| R13S | 99.4 | 6.4 | 88 | 90 | 42 |
| R14S | 99.3 | 3.4 | 96 | 85 | 50 |
| A Supra | 99.4 | 1.6 | 96 | 81 | 82 |

Activated Carbon Specifications

| Activated Carbon | Activated Carbon | Ash Content | Activation | Special Treatment |
|---|---|---|---|---|
| R11S | Mineral | 14 % | Steam | No |
| R12S | Lignite | not determined | Steam | No |
| R13S | Peat | 8% | Steam | Acid washed |
| R14S | Peat | 8% | Acid ($H_2SO_4$) | No |
| A Supra | Coconut Shells | 3% | Steam | No |

The water flow rate for R10S media is 2 gallons water/min/square foot with a differential pressure of 1.5 p.s.i.

This example shows that the choice of activated carbon used can have a strong effect on the IAD's impact on biological activity.

Notes: The A Supra grade was prepared to the same specifications as the R1xS series; only the carbon was changed. An increase in aPTT indicates removal of clotting factors. I, VIII, and IX refer to particular clotting factors. All values are relative to post-photochemical treatment. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen was assayed by HPLC and clotting factors and times were assayed by an automated coagulation analyzer.

## EXAMPLE 6

*Effect of Particle Size on Low Molecular Weight Compound Removal and Biological Activity.* This example demonstrates that the size of the adsorbent particles used in the IAD can have an important effect on the degree of low molecular weight compound removal and measures of biological activity.

Photochemically treated plasma was similarly prepared as in Example 5. Dowex Optipore L493 was ground with a Estro Model 480 grinder and sieved with approximately 100 µm and 50 µm sieves to generate two classes of particles, those between 50 µm and 100 µm and those less than 50 µm. ZetaPlus-like filters were prepared containing either of these two classes of particles according to Cuno R1xS specifications. Photochemically treated plasma (325 mL) was pumped through each pad at 20 mL/min. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethylpsoralen and clotting factors were analyzed as in Example 5.

| Particle Size (µm) | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Yield | VIII % Yield | IX % Yield |
|---|---|---|---|---|---|
| 100 >> | 96.1 | 0.9 | 97 | 94 | 86 |

| | | | | | |
|---|---|---|---|---|---|
| 50 | | | | | |
| < 50 | 99.2 | 2.2 | 83 | 91 | 76 |

With this particular adsorbent, the smaller particle size resulted in substantially better removal but had a larger impact or biological activity, as measured by clotting activity. This illustrates the trade-off that is often seen with selecting a particle size for the adsorbent particle.

## EXAMPLE 7

*Effect of Adsorbent Loading on Low Molecular Weight Compound Removal and Biological Activity.* This example compares the effect of changing the mass fraction of the active component on reducing the concentration of a small organic compound and the tradeoff with the fluid's biological function.

Photochemically treated plasma was similarly prepared as in Example 5. A Supra pads were prepared with the standard R1xS loading of approximately 60% Carbon and a lower loading level of 30%. Approximately 230 mL plasma was pumped through each 90 mm disc. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen and clotting factors were analyzed as in Example 5.

| A Supra Loading (%) | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Yield | VIII % Yield | IX % Yield |
|---|---|---|---|---|---|
| 61.5 | 99.4 | 1.3 | 93 | 93 | 77 |
| 30.8 | 99.4 | 0.9 | 99 | 90 | 91 |

This demonstrates the unexpected result that by reducing the loading of adsorbent particles, it is sometimes possible to reduce the IAD's effect on biological activity, while still substantially reducing the concentration of the low

molecular weight compound. Overall capacity of the IAD will be reduced, but as long as the IAD is operated at significantly less than the theoretical capacity for the low molecular weight compound, this does not matter.

## EXAMPLE 8

*Effect of Using a Hemocompatible Coating.* This example demonstrates that in some cases, treating the media with a hydrophilic polymer can have benefits to biological function.

Photochemically treated plasma was similarly prepared as in Example 5. One 90 mm R14S pad was flushed overnight with a 50 mg/mL solution of polyhydroxyethymethacrylate (pHEMA) in 95% ethanol dried in a 70°C oven until there was no additional weight change. The second pad was similarly flushed with 95% ethanol without pHEMA and dried. Approximately 325 mL plasma was pumped through the discs at 5 mL/min. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen and clotting factors were analyzed as in Example 5.

| Coating | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Yield |
|---|---|---|---|
| None | 99.6 | 4.1 | 98 |
| pHEMA | 99.1 | 2.5 | 103 |

Though the coating showed benefits to the biological activity of the biological composition, in this case, the clotting activity of plasma, coating had an adverse effect on the ability of the IAD to reduce the concentration of the low molecular weight compound used in this example, . 4'-(4-amino-2-oxa)butyl-4,5',8-trimethylpsoralen. This illustrates the trade-offs that are often seen when selecting a coating or surface treatment.

EXAMPLE 9

*Effect of Flow Rate on Low Molecular Weight Compound Removal and Biological Activity.* This example demonstrates that flowrate can have an effect on the reduction in concentration of the small organic compound.

Photochemically treated plasma was similarly prepared as in Example 5. A Supra pads were prepared with the standard R1xS loading of approximately 60% carbon and 325 mL of the treated plasma was pumped through each of the 47 mm diameter discs at three different flowrates. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen and clotting factors were analyzed as in Example 5.

| Flowrate (mL/min) | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Yield | VIII % Yield | IX % Yield |
|---|---|---|---|---|---|
| 10 | 99.0 | 1.3 | 98 | 83 | 80 |
| 15 | 98.8 | 1.5 | 99 | 85 | 86 |
| 20 | 98.7 | 1.4 | 97 | 89 | 86 |

Though increasing the flow rate showed a slight decrease in the degree of removal of low molecular weight compound, it had a more substantial benefit to the biological activity, as seen by the higher yield of factor VIII and factor IX clotting activity. This shows that adjusting the flux of the biological composition through the IAD can confer selectivity for low molecular weight compound reduction over effects on biological activity.

EXAMPLE 10

*Effect of Fluid Volume on Low Molecular Weight Compound Removal and Biological Activity.* This example demonstrates that fluid volume can also have

an important effect on conferring selectivity for low molecular weight compounds over mediators of biological activit

Photochemically treated plasma was similarly prepared as in Example 5. ZetaPlus-like filters were prepared with ground Dowex Optipore L493 with particles less than 50 μm instead of powdered activated carbon according to the Cuno R1xS specifications. Plasma was pumped through the filter at 20 mL/min and samples were taken at 180 mL and 325 mL. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen and clotting factors were analyzed as in Example 5.

| Plasma Volume (mL) | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Yield | VIII % Yield | IX % Yield |
|---|---|---|---|---|---|
| 180 | 99.3 | 4.1 | 74 | 89 | 66 |
| 325 | 99.2 | 2.2 | 83 | 91 | 76 |

Thus, by increasing the fluid volume treated, additional selectivity can be conferred on low molecular compound removal over reduction in biological activity. There is a limit to this of course, as the capacity of the IAD for of the low molecular weight compound is approached, selectivity will again decrease.

## EXAMPLE 11

*Use of Sintered Media.* Ninety millimeter diameter by 1/4" thick discs were fabricated by Porex. The discs contained various weight fractions of finely ground Dowex Optipore L493 with particle sizes between 20 μm and 100 μm, and small particles of ultra high molecular weight polyethylene (grade UF220), which were then sintered together. Photochemically treated plasma was similarly prepared as in Example 5 and 200 mL of plasma was pumped through each disc at

16-18 mL/min. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen and clotting factors were analyzed as in Example 5.

| Weight Percent Adsorbent | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Yield | VIII % Yield | IX % Yield |
|---|---|---|---|---|---|
| 25 | 97.8 | 0.5 | 93 | 95 | 78 |
| 35 | 99.0 | 1.2 | 90 | 94 | 74 |
| 50 | 99.3 | 1.2 | 91 | 80 | 76 |

As was seen using a fibrous matrix with activated carbon in example 7, changing the fraction of adsorbent in the IAD also has an effect both on low molecular weight compound removal and biological activity when using a sintered matrix. In this case, the 25% formulation does not give as good removal of 4'-(4-amino-2-oxa)butyl-4,5',8-trimethylpsoralen, as the 35% and 50% formulations. The 50% formulation has a greater loss in factor VIII activity than the other formulations. Of the three medias, the 35% formulation confers the highest selectivity of removal of low molecular weigh compound over reduction in biological activity.

## EXAMPLE 12

This example describes the effect of increasing the mass of adsorbent by increasing the diameter of the filter at constant thickness.

Photochemically treated plasma was similarly prepared as in Example 5. Approximately 325 mL of treated plasma was pumped through 90 and 47 mm diameter R03S grade discs at 5 mL/minute. 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen and clotting factors were analyzed as in Example 5.

| Disc Diameter (mm) | 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen % Removed | PTT Increase (s) | I % Retained |
|---|---|---|---|
| 47 | 99.4 | 2.1 | 96 |
| 90 | 99.6 | 3.5 | 77 |

Increasing the diameter of the adsorbent material while keeping the same thickness and flow rate has the equivalent effect of reducing the flux and reducing the volume treated per unit cross sectional area of filter. Although both of these effects tend to increase adsorption of low molecular weight compounds, they often increase the adsorption of mediators of biological activity, even more. In this example we see that slightly more S-59 was adsorbed with the larger diameter adsorbent material, but substantially more factor I activity was lost.

## EXAMPLE 13

*Flow Studies Using Several Forms of Activated Carbon Media.*

Experiments were carried out to investigate removal of 5-[(β-carboxyethyl)-amino]acridine and GSH from PRBCs using a flow device. PRBCs (Erythrosol, glucose, 63% HCT) were dosed with 300 μM of a degradable 5-[(β-carboxyethyl)amino]acridine derivative and 3 mM GSH, and held at room temperature without agitation for 20 hours prior to flow through devices. The flow compound adsorption device media consisted of various forms of activated carbon, as either a composite carbon/cellulose disk, or a carbon fiber felt. The media were sealed into a 90 mm diameter polycarbonate housing (Cuno, Meridien, CT). The dosed PRBCs were pumped (Gilson Minipuls, Middleton, WI) through the media at a flow rate of 5 mL/min, and collected in a PL146 plastic container (Baxter Healthcare). The results of this study are shown in Table 1.

**Table 1.** Flow study results using various activated carbon media.

| Media | Description | Residual Acridine* (μM) | Residual GSH (mM) | % Hemolysis |
|---|---|---|---|---|
| CUNO | A Supra/Cellulose disk (30% loading) | 26.04 | 0.94 | 4.83 |
| CUNO 95-1 | A Supra/Cellulose disk (70% loading) | 25.08 | 0.68 | 7.56 |
| CUNO 95-2 | A Supra/Cellulose disk (70% loading) | 19.22 | 0.11 | 5.15 |
| Cellulo | A Supra/Cellulose disk (60% loading) | 23.62 | 0.79 | 5.19 |
| FPI | A Supra/Cellulose disk (70% loading) | 20.78 | 0.20 | 5.27 |
| Ertel | A Supra/Cellulose disk (60% loading) | 25.13 | 0.14 | 7.17 |
| Actitex | Activated C felt-1 layer-162 $g/m^2$ | 51.36 | 6.14 | 1.27 |
| Lantor | Activated Carbon felt | 30.90 | 4.27 | 1.78 |
| Ultrasorb | Activated Carbon felt (200 $g/m^2$) | 77.79 | 5.13 | 1.06 |
| Actitex | Activated Carbon felt-3 layers (162 $g/m^2$) | 28.75 | 5.58 | 1.37 |
| Lydall | Activated Carbon felt | 39.69 | 5.04 | 1.18 |
| MN-200 | MN-200/Cellulose disk (70% loading) | 87.87 | 6.44 | 1.11 |

## EXAMPLE 14

*Flow Compound Adsorption Devices (CUNO media) vs. Batch Compound Adsorption Devices (AQF media).* Studies were performed which compared 5-[(β-carboxyethyl)amino]acridine and GSH removal in flow versus batch compound adsorption devices. The flow device consisted of a cellulose/Norit A Supra (Norit Americas, Inc. (Atlanta, GA)) carbon disk enclosed in a 90 mm housing. There were two separate batch devices: one consisted of fiberized Pica G277 activated carbon (AQF 500 $g/m^2$); the other consisted of fiberized Purolite

MN-200 (AQF 312 g/m$^2$). Following dosing with 300 μM of a degradable 5-[(β-carboxyethyl)-amino]acridine derivative and 3 mM GSH, PRBCs were pumped through the cellulose/carbon media at a flow rate of 2 mL/Min, after which 5-[(β-carboxyethyl)-amino]acridine and GSH levels dropped 75 and 88%, respectively, to 24 μM and 0.71 mM in the PRBC supernatant. The flow device exposed PRBCs were then transferred to the 6 g MN-200 batch device, which decreased 5-[(β-carboxyethyl)-amino]acridine and GSH levels by an additional 5 and 1%, reaching 20 μm and 0.67 mM over 24 hours. Exposure of PRBCs to a 7 g Pica G277 batch device alone resulted in a drop in 5-[(β-carboxyethyl)-amino]acridine and GSH levels by 92 and 54% to concentrations of 8 μM and 3 mM. Therefore, one pass through the flow device was more effective in removing GSH than exposure to the carbon batch device for 24 hours. The batch device alone, however, was more effective in removing 5-[(β-carboxyethyl)-amino]acridine than the flow and MN-200 devices combined. Flow through the device did not seem to have an adverse effect on PRBC ATP concentration. K+ levels in PRBCs were lower after exposure to the flow device as compared to a 24 hour carbon batch device exposure.

## EXAMPLE 15

This example describes the typical performance of an immobilized adsorbent device in a flow mode on plasma using adsorbent media composed of 30% Norit A Supra carbon (Norit Americas, Atlanta, GA) and manufactured by Cuno (Meriden, CT) previously described in an earlier example.

IADs were assembled using 90 mm Cuno 30% Norit A Supra impregnated R10SP media in series with 90 mm diameter Memtec hydroxypropylcellulose coated polysulfone membrane with 5 μm pores.

To complete disposable manufacture, a 1 LPL2410 bag was docked to 1/8" OD tubing and the tubing was then attached to the outlet of the IAD such that

the distance from the midline of the IAD to the bag was 40cm. The same sized tubing was attached to the inlet of the SRD such that when the illumination bag is docked to it, the distance from the midline to the bag would be 30 cm.

4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen was added to 500 mL of plasma to a final concentration of approximately 150 μM, and the plasma was illuminated to 6.3 J/cm2 UVA to inactivate pathogens. The post-illumination 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen concentration was approximately 82 μM.

Table 2 shows measures of clotting factor activity and 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen levels after having been treated by the IAD relative to values beforebeing passed through the IAD. The experiment was repeated three times. Means and standard deviations are reported. Treatment time averaged 14 minutes. It is apparent that there is virtually no change in factors I, II, V, VII, X or measures of aggregate clotting activity, the prothrombin time (PT) and activated partial thromboplastin time (aPTT) and very small changes in factors XI and XII. Factors VIII and IX show somewhat larger changes but these are acceptable, especially in light of the prescription of recombinant proteins for their deficiency. The very selective nature of the device should be noted in that it retains virtually all the clotting activity while allowing only 0.9% of the 4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen to pass through.

**Table 2**

| | |
|---|---|
| PT(s) | 0.0 ± 0.1 |
| aPTT(s) | 0.7 ± 0.1 |
| I (%) | 98 ± 2 |
| II (%) | 99 ± 1 |
| V(%) | 101 ± 2 |
| VII(%) | 99 ± 2 |
| VIII (%) | 86 ± 1 |

| | |
|---|---|
| IX(%) | 82 ± 3 |
| X (%) | 103 ± 4 |
| XI (%) | 94 ± 4 |
| XII(%) | 95 ± 5 |
| S-59(%) | 0.9 ± 0.1 |

EXAMPLE 16

*Reduction of Activated Complement by Adsorbent Medias.* This study demonstrates the removal of biological response modifiers by cellulose media impregnated with activated carbon and porous plastic media impregnated with ground polystyrene/divinylbenzene adsorbent.

Zymosan (Sigma Chemical Company; St. Louis, MO), a potent activator of the complement cascade, was added to plasma at a concentration of 10 mg/mL. The plasma was incubated with mild shaking at 37 °C for 1 hour. The plasma was then centrifuged and the supernatant saved to get rid of the solid zymosan. Approximately 20 mL of this supernatant was added to each of two 600 mL units of plasma and a sample from each unit was taken for C3a and SC5b-9 analysis. One of these units was pumped at 40 mL/min through a 90 mm disc of carbon impregnated cellulose media (Cat. #2640ASP, Cellulo, Inc.; Fresno, CA). The other unit was pumped at the same rate through sintered media prepared as in Example 11 (Porex Technologies; Fairburn, GA). The filtrate from each unit was sampled for C3a and SC5b-9 analysis.

Complement assays were performed using Quidel assay kits.

| | [C3a] (ng/mL) | [SC5b-9] (ng/mL) |
|---|---|---|
| Pre-filtration | 1597 | 5210 |
| Filtration with carbon/cellulose media | 521 | 1747 |
| Filtration with sintered media | 68 | 1592 |

Biological response modifiers can also be removed from biological compositions, as the previous table indicates.

EXAMPLE 17

*Comparison of Carbon Fiber Medias with Carbon Impregnated Cellulose.* This example compares the use of carbon fiber medias disclosed in US Patent 5,660,731 with medias disclosed herein.

Photochemically treated plasma was similarly prepared as in Example 5. Approximately 175 mL of the treated plasma was pumped through each of the medias at about 11 mL/min. S-59 and clotting factors were analyzed as in Example 5.

| Media | Total Thickness (in.) | Number of Layers | S-59 Reduction (%) |
|---|---|---|---|
| Kynol CAN-211-20 | 0.203 | 5 | 74.9 |
| Kuractive FT300-20 Felt | 0.234 | 7 | 37.2 |
| Actitex FC1201 | 0.328 | 7 | 44.7 |
| Cuno A Supra | 0.250 | 1 | 99.1 |

Sources of medias: Kynol (American Kynol, Inc.; Pleasantville, NY); Kuractive (Kuraray Chemical Co.; Bizen City, Japan); Actitex (Pica USA; Columbus, OH); Cuno A Supra (Cuno, Inc.; Meridien, CT).

As the above table shows, using carbon fiber medias to remove low molecular weight compounds from biological compositions as disclosed in US patent #5,660,731 often does not adequately reduce the concentration of low molecular weight compounds in biological compositions compared to adsorbent medias disclosed herein.

EXAMPLE 18

*Effect of Adsorbent Pore Size Distribution on Biological Activity.* This example demonstrates that the pore size distribution of the adsorbent contained in the IAD can have an important effect on the IAD's ability to maintain biological activity.

Ninety millimeter diameter by 1/4" thick porous plastic discs were fabricated by Porex Technologies (Fairburn, GA). The discs were a sintered mixture of approximately 50% by weight of about 25 μm diameter particles of ultra high molecular weight polyethylene and 50% of one of Purolite's (Bala Cynwyd, PA) non-functionalized Hypersol-Macronet adsorbents that were ground by Porex such that more than 90% of the particles (by weight) were between 60 μm and 160 μm in diameter. Photochemically treated plasma was prepared by adding a solution of S-59 to each of three approximately 600 mL units of plasma for an S-59 concentration of about 150 μM, and illuminating each unit with 6.3 J/cm2 UVA as previously described. The treated units were pooled and approximately 600 mL of the pool was pumped through each of the discs at about 40 mL/min. S-59 and clotting factors were analyzed as in Example 5. Surface area was analyzed by equilibrated-step mercury intrusion porosimetry (Micromeritics, Norcross, GA).

| Adsorbent | Cumulative Surface Area Associated with Pores > 3 nm in diameter ($m^2/g$) | Cumulative Surface Area Associated with Pores > 20 nm in diameter ($m^2/g$) | Cumulative Surface Area Associated with Pores > 40 nm in diameter ($m^2/g$) |
|---|---|---|---|
| MN-200 | 188 | 55 | 42 |
| MN-250 | 172 | 28 | 7 |
| MN-270 | 165 | 14 | 3 |

| Adsorbent | S-59 | aPTT increase | Factor IX | Factor XI |
|---|---|---|---|---|

| | Removed (%) | (s) | activity (% retained) | activity (% retained) |
|---|---|---|---|---|
| MN-200 | 99.5 | 5.8 | 85 | 55 |
| MN-250 | 99.5 | 2.0 | 82 | 77 |
| MN-270 | 99.4 | 0.5 | 100 | 91 |

The choice among these three particular adsorbents used in the IADs had a strong effect on biological activity, as measured by aPTT, Factor IX activity, and Factor XI activity, but did not have a strong effect on the degree of reduction of the low molecular weight compound used in this example, S-59.

Since the three adsorbents used in the IADs in this example, MN200, MN250, and MN270, differ chiefly in the pore size at which each begins to have appreciable surface area and not substantially in their surface chemistry, a conclusion consistent with the above results is that the surface area accessible by the low molecular weight compound is similar among the three adsorbents, resulting in a similar degree of S-59 reduction for each IAD. In addition, it is likely that the better biological activity shown by the IADs containing adsorbents with less surface area associated with larger pores is due to the larger pores allowing certain mediators of the measured biological activity that have a tendency to adsorb to the adsorbents' surfaces, namely factor IX and factor XI in this example, to adsorb or inactivate on the larger pores' surfaces, while smaller pores exclude those mediators from their surfaces.

## EXAMPLE 19

This example demonstrates the utility of using IADs for removing low molecular weight compounds such as viral inactivating agents (psoralen) or biological response modifiers (activated complement-C3a) from whole blood. Whole blood was pre-treated with cellulose acetate membrane to cause complement activation as may be observed in hemodialysis when using cellulose acetate membranes. Hemoperfusion to remove activated complement and added

psoralen was simulated by returning the effluent from the hemoperfusion device to a mixed pool of whole blood (see Figure 13).

Two units of ABO-matched whole blood were obtained from the Sacramento Blood Center (Sacramento, CA). The units were maintained at room temperature following donation. The two units were transferred to two PL2410 plastic storage containers (Baxter Healthcare, Deerfield, IL) containing 4 pieces of Millipore RA type membrane (47 mm, Millipore, Marlborough, MA). The whole blood was incubated for 24 hours at room temperature with the cellulose acetate membranes (Millipore RA) to induce complement activation. Psoralen (150 μM S-59 (4'-(4-amino-2-oxa)butyl-4,5',8-trimethyl psoralen)) was added to each whole blood unit immediately before hemoperfusion was initiated.

The IAD media (300 g/m$^2$ MN-200, AQF) was cut into circular disks with a diameter of 47 mm. Disks were sealed in a 47 mm diameter polycarbonate housing with a stainless steel support screen (Cuno Inc., Meridien, CT). Tubing (3 mm ID PharMed tubing) was attached to the housing. The tubing was loaded in a peristaltic pump (Masterflex) and the system was calibrated to deliver a flow rate of 75 mL/min. The tubing inlet and outlet were attached to a 600 mL beaker (Nalgene) which was placed on a stir plate (see Figure 13). The whole blood which was contained in the 500 mL beaker was gently agitated with a Teflon-coated stir bar throughout the study to simulate the mixing that would occur in the subject's body.

The entire assembly was rinsed with a solution containing 174 USP units heparin (sodium salt, grade 1-A, 174 USP units/mg, Sigma Chemical Co.) per mL of saline. The saline was purged from the IAD assembly before introducing the whole blood. The unit of whole blood (500 mL) was added to the beaker. Agitation was slowly increased until mixing of the blood was apparent. The flow of whole blood was initiated. Whole blood was flowed from the bottom of the IAD up as indicated in Figure 13. Samples of whold blood were taken from the beaker at 15 minute intervals. Cell counts were performed immediately using a Baker cell counter (manufacturer). Samples were centrifuged in a

microcentrifuge (10,000 rpm, 5 min) and the supernatant was removed and immediately frozen for later analysis.

Samples of supernatant were thawed and were analyzed for residual levels of psoralen using reversed-phase HPLC. In addition, levels of activated complement fragment C3a were determined by using an ELISA (Quidel, San Diego, CA). Finally, levels of hemolysis were determined as described previously.

The results from the cell counts, hemolysis measurement, psoralen measurement, and C3a measurement are summarized in Table 3. In general, removal of both psoralen and C3a were demonstrated. In addition, whit blood cell (WBC) and red blood cell (RBC) counts remained essentially constant throughout the study. Platelets counts did show a downward trend during study and there was a slight increase in hemolysis. Further studies with fresh whole blood will be required to demonstrate whether losses in platelets could be attributed to the initial room temperature incubation that the whole blood was subjected to.

The advantages of using immobilized adsorbents in hemoperfusion devices include: 1) the ability to independently control adsorbent particle size and pressure drop-especially important at high flow rates or for small particle adsorbents; 2) the ability to control particle attrition by immobilizing the adsorbent particles thereby minimizing physical interactions; 3) the ability to minimize small particle contamination and shedding from the device by immobilizing adsorbent particles; 4) the ability to maintain a uniform and stable adsorbent bed.

Table 3. Results from Whole Blood Hemoperfusion Study.

| Time (min) | WBC Count ($x10^3/\mu L$) | RBC Count ($x10^6/\mu L$) | Platelet Count ($x10^3/\mu L$) | Hemolysis (%) | Residual Psoralen ($\mu M$) | Residual C3a (ng/mL) |
|---|---|---|---|---|---|---|
| 0 | 4.5 | 3.72 | 228 | 0.22 | 150.0 | 959 |
| 18 | 4.3 | 3.63 | 224 | 0.29 | 105.8 | 707 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 30 | 4.4 | 3.79 | 217 | 0.32 | 92.1 | 716 |
| 46.5 | 4.2 | 3.69 | 204 | 0.32 | 77.7 | 573 |
| 60 | 3.9 | 3.79 | 203 | 0.38 | 68.8 | 537 |
| 75 | 4.1 | 3.73 | 181 | 0.39 | 58.2 | 477 |
| 90 | 4.1 | 3.70 | 186 | 0.42 | 53.1 | 547 |

5

## CLAIMS

1. A method of reducing the concentration of a biological response modifier in a biological composition, wherein the method substantially maintains a desired biological activity of the biological composition, comprising treating the biological composition with a device, wherein the device comprises an inert matrix containing highly adsorbent particles, and wherein the adsorbent particles range from about 1 μm to about 200 μm in diameter, and wherein the device is for use in a flow process.

2. A method according to claim 1, wherein the biological response modifier is activated complement.

3. A method according to claim 1, wherein the biological composition is plasma.

4. A method according to claim 1, wherein the adsorbent material has a length less than three times the width.

5. A method according to claim 1, wherein the adsorbent particles comprise a hypercrosslinked polystyrene network.

6. A method according to claim 1, wherein the adsorbent particles are activated carbon particles, and wherein the activated carbon particles have a surface area greater than about 1200 $m^2/g$.

7. A method according to claim 6, wherein the activated carbon particles are formed by steam activation of coconut shells.

8. A method according to claim 1, wherein the inert matrix of the device is a fiber network composed of cellulose.

9. A method according to claim 1, wherein the inert matrix is a particulate network formed by sintering together particles of ultra-high molecular weight polyethylene with particles of hypercrosslinked polystyrene networks.

10. A method according to claim 1, wherein the method further reduces the concentration of a psoralen derivative, an acridine derivative, a dye or a quencher in the biological composition.

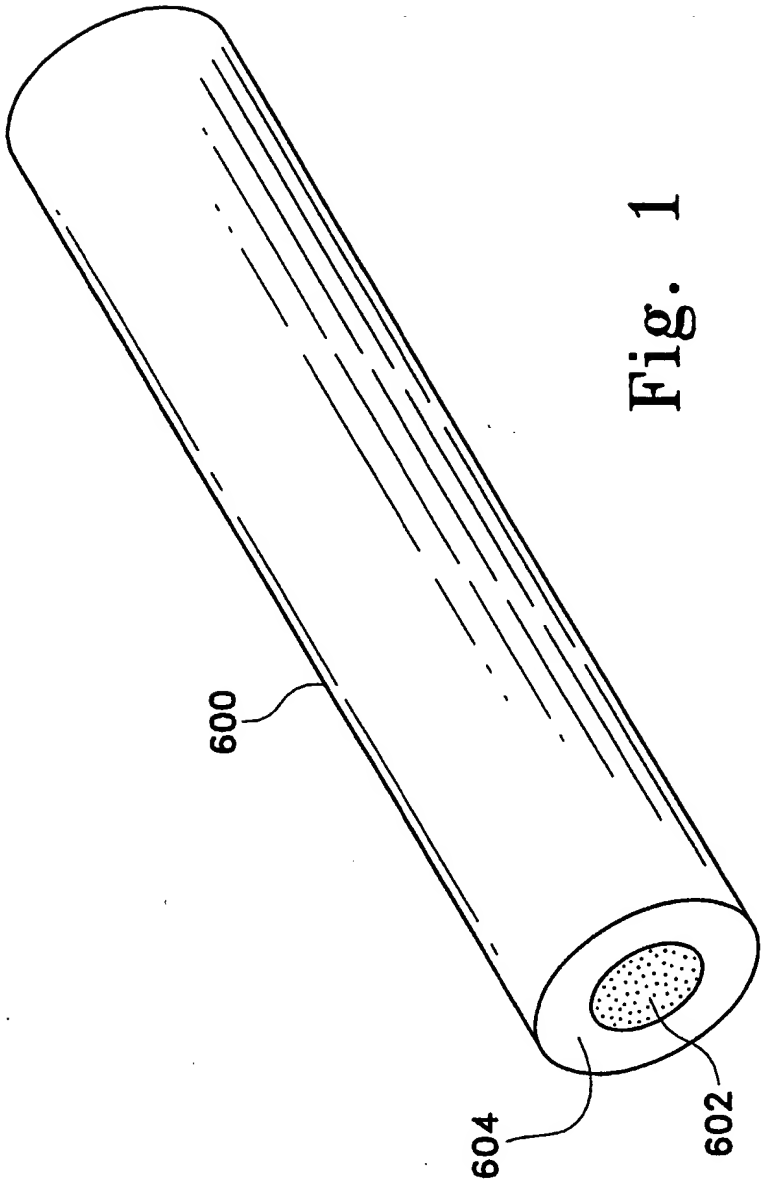



Fig. 1

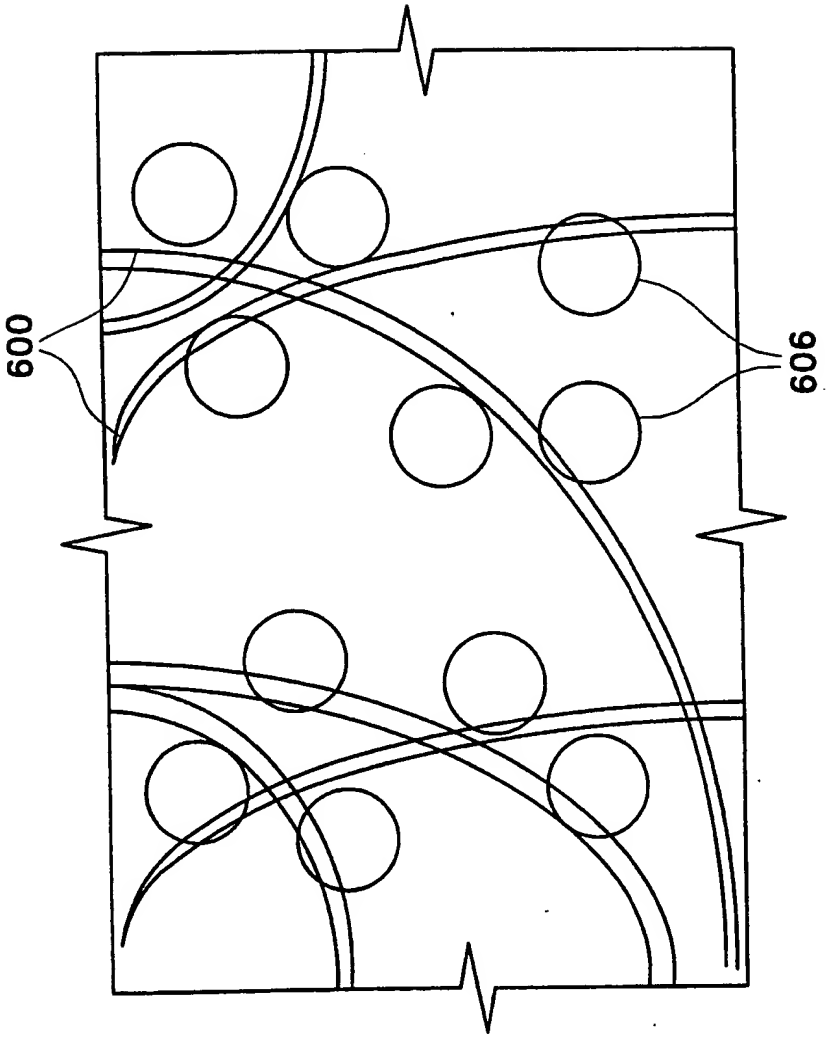



Fig. 2

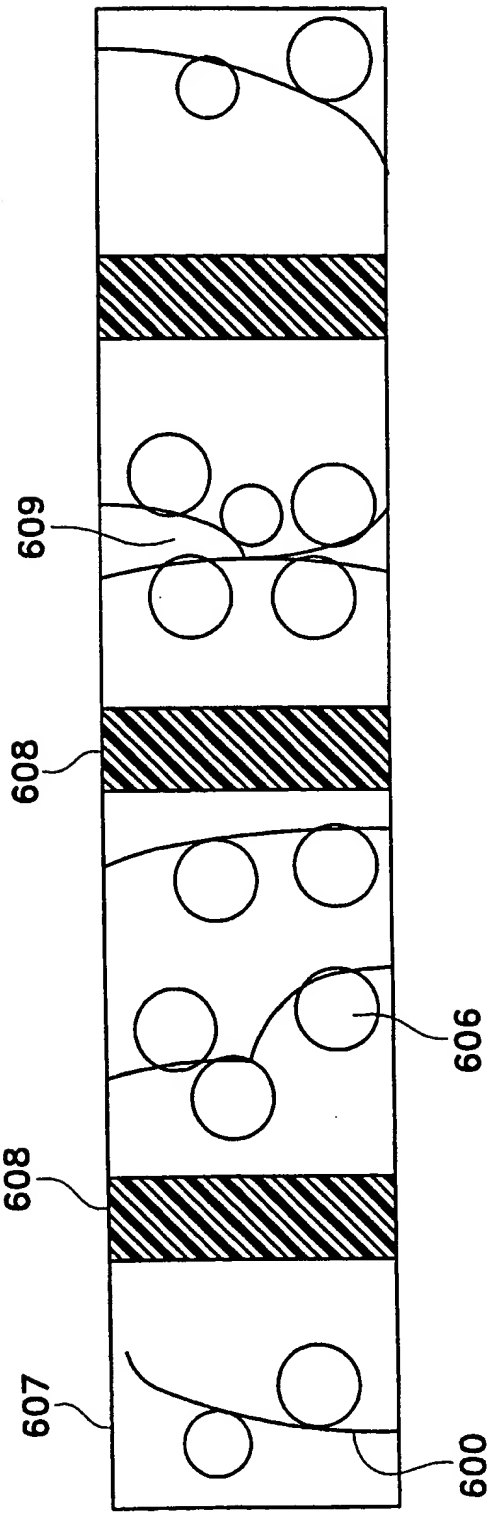



Fig. 3

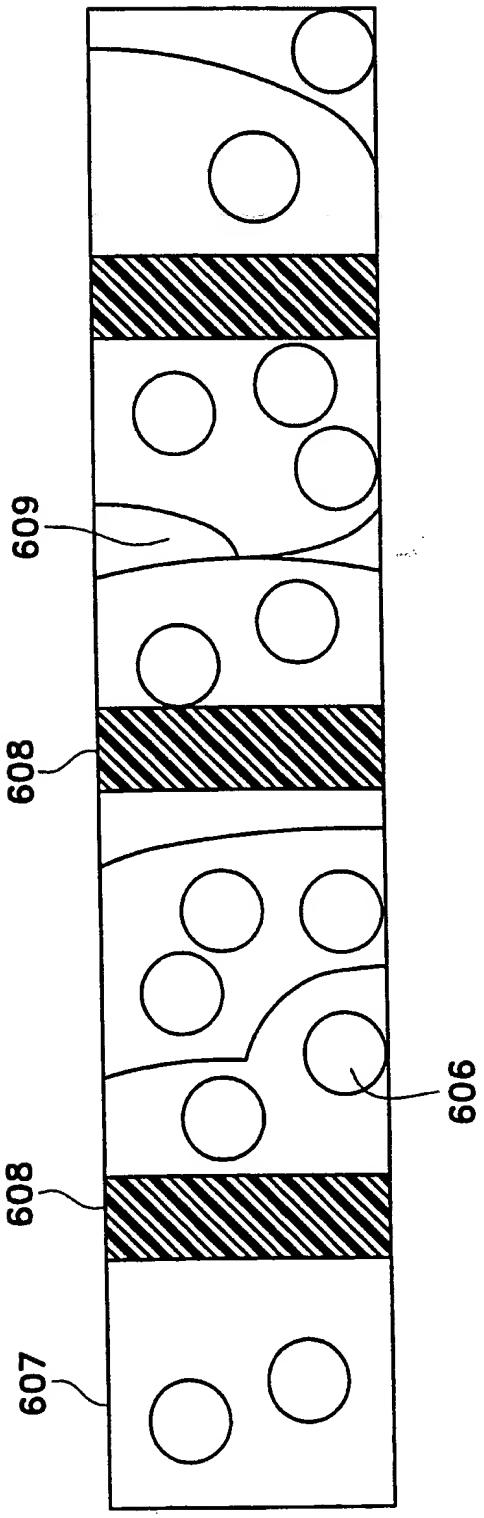



Fig. 4

Fig. 5

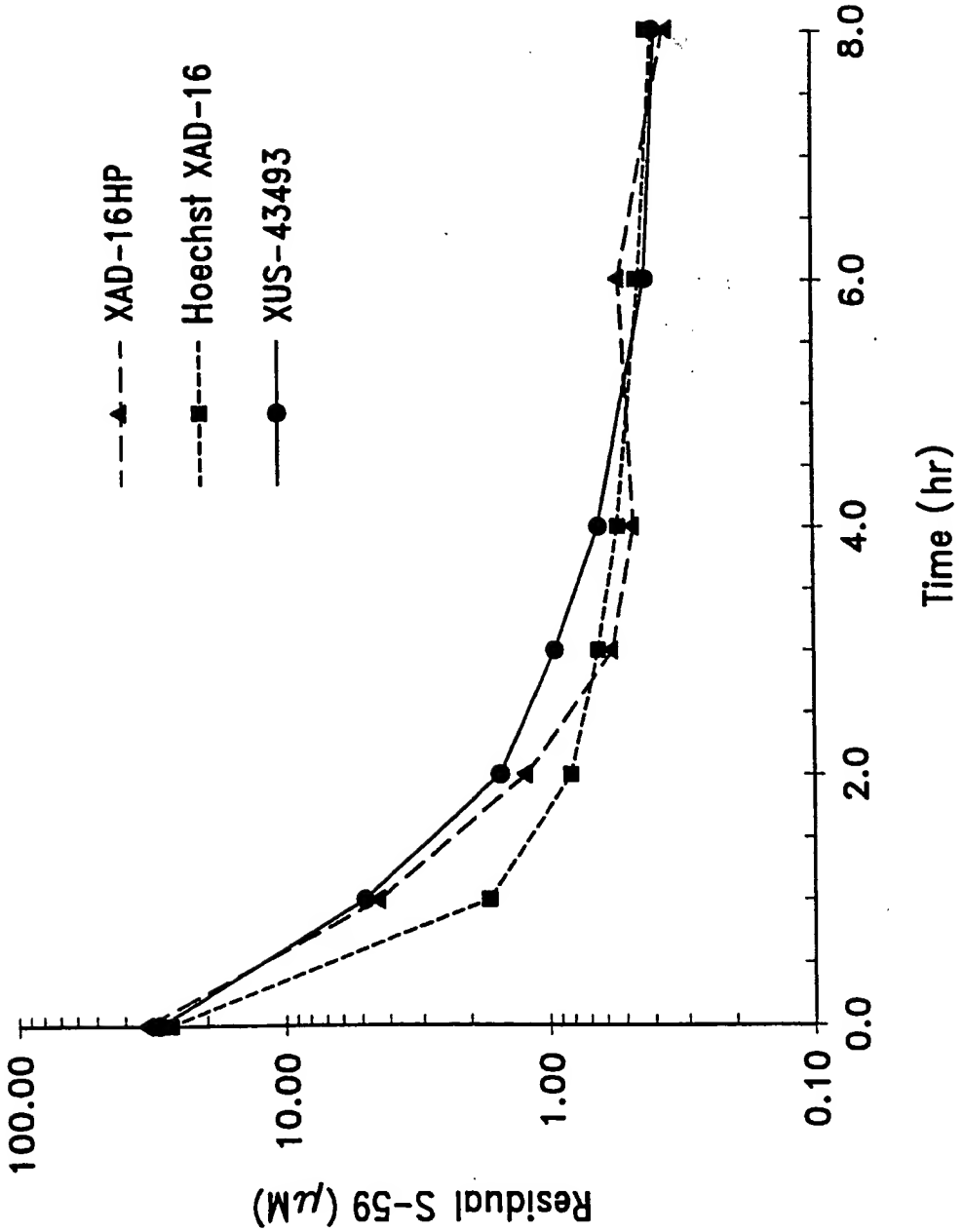

XAD-16HP
Hoechst XAD-16
XUS-43493
Residual S-59 (μM)
100.00
10.00
1.00
0.10
0.0
2.0
4.0
6.0
8.0
Time (hr)

Fig. 6

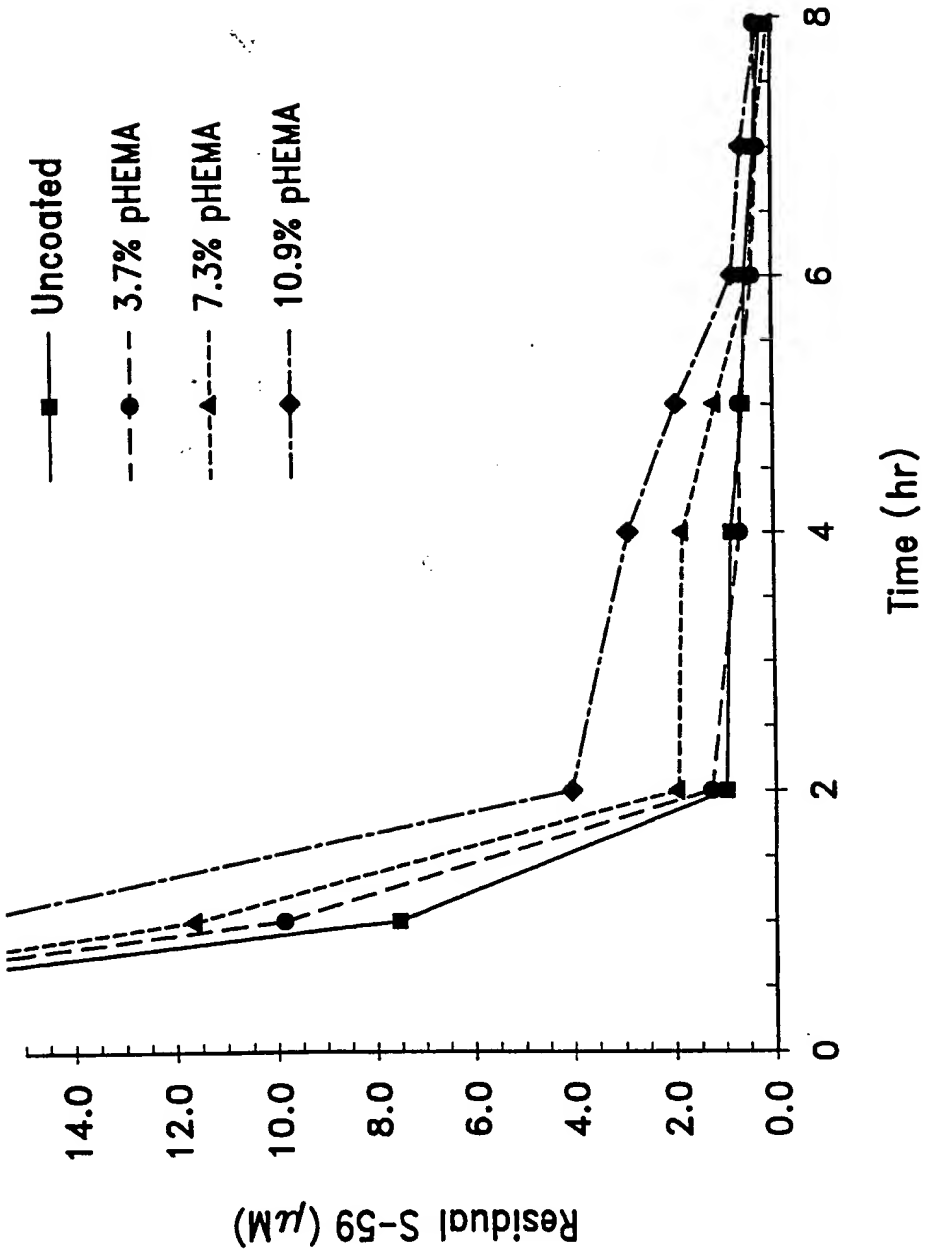

Uncoated
3.7% pHEMA
7.3% pHEMA
10.9% pHEMA
Residual S-59 (μM)
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Time (hr)
0
2
4
6
8

Fig. 7

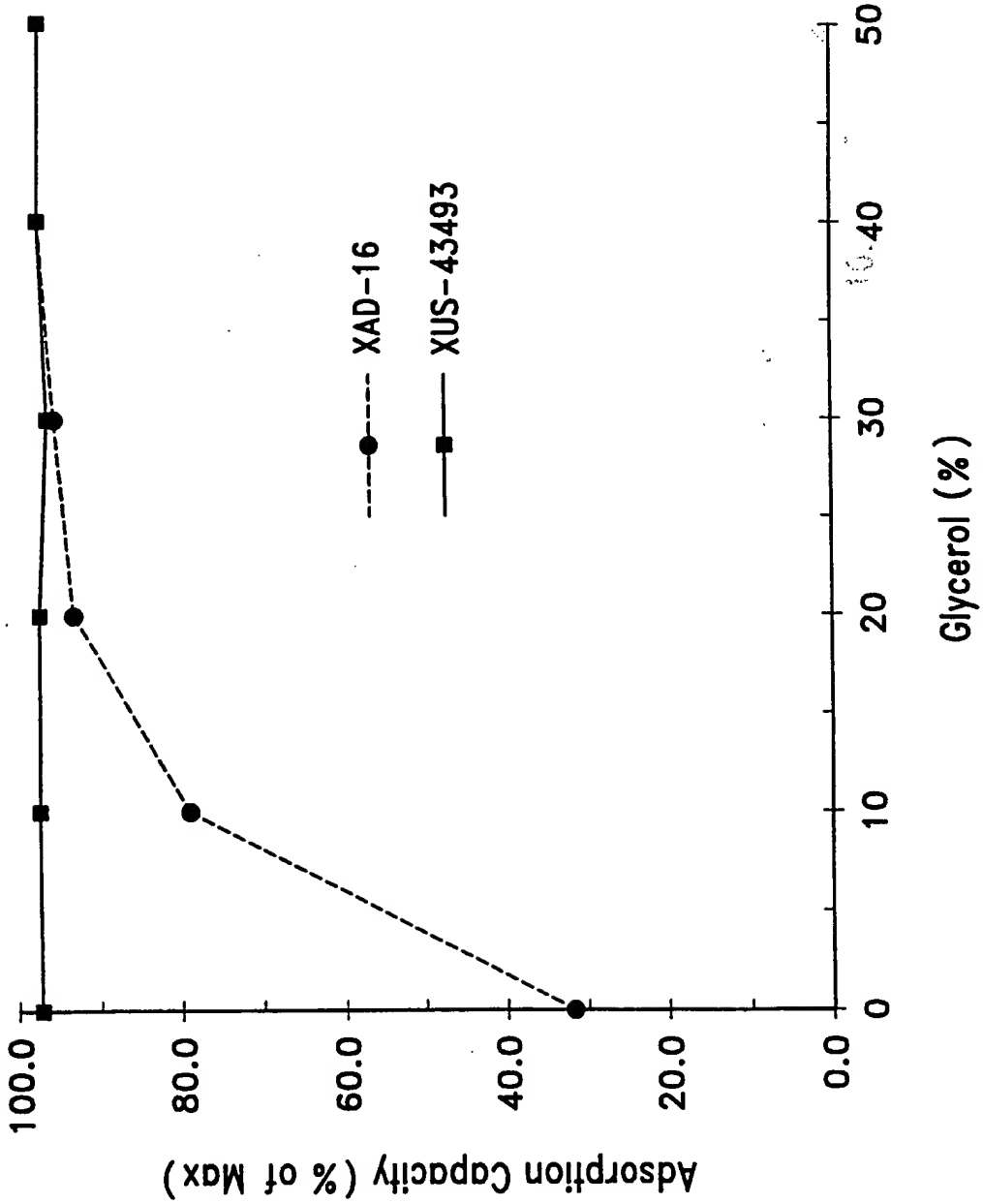

Adsorption Capacity (% of Max)
100.0
80.0
60.0
40.0
20.0
0.0
0
10
20
30
40
50
Glycerol (%)
XAD-16
XUS-43493

Fig. 8

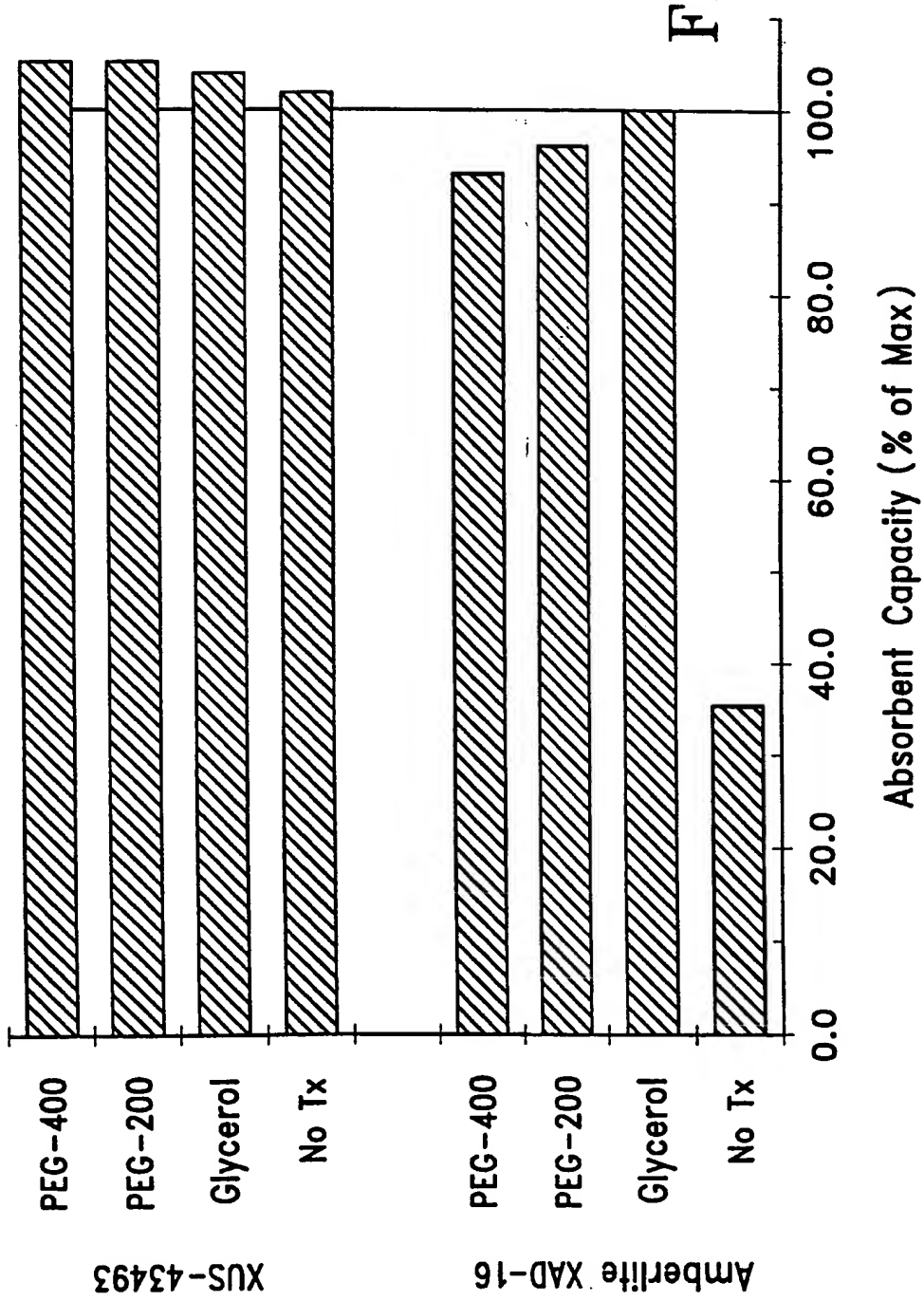

Absorbent Capacity (% of Max)
0.0
20.0
40.0
60.0
80.0
100.0
PEG-400
PEG-200
Glycerol
No Tx
PEG-400
PEG-200
Glycerol
No Tx
XUS-43493
Amberlite XAD-16

Fig. 9

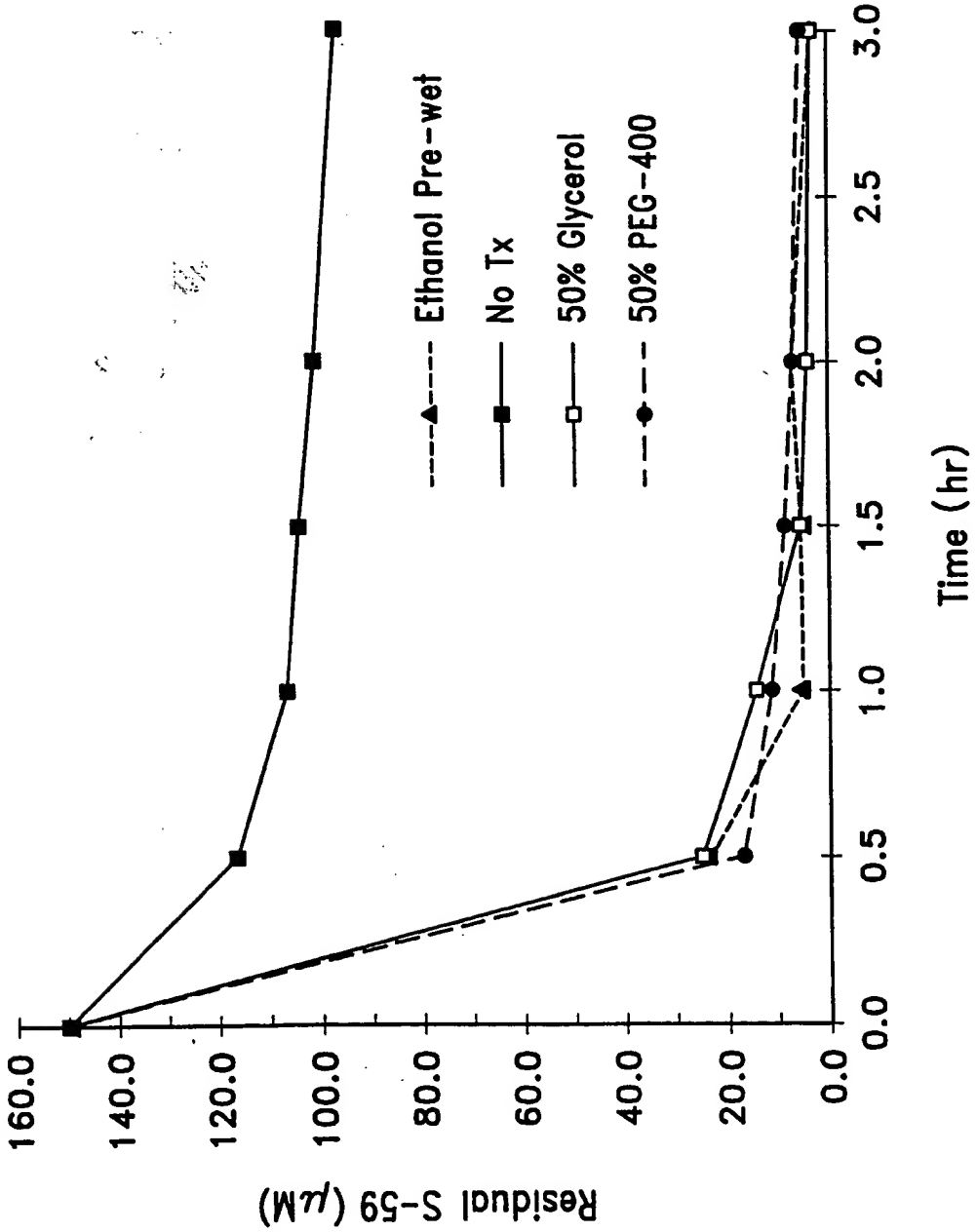
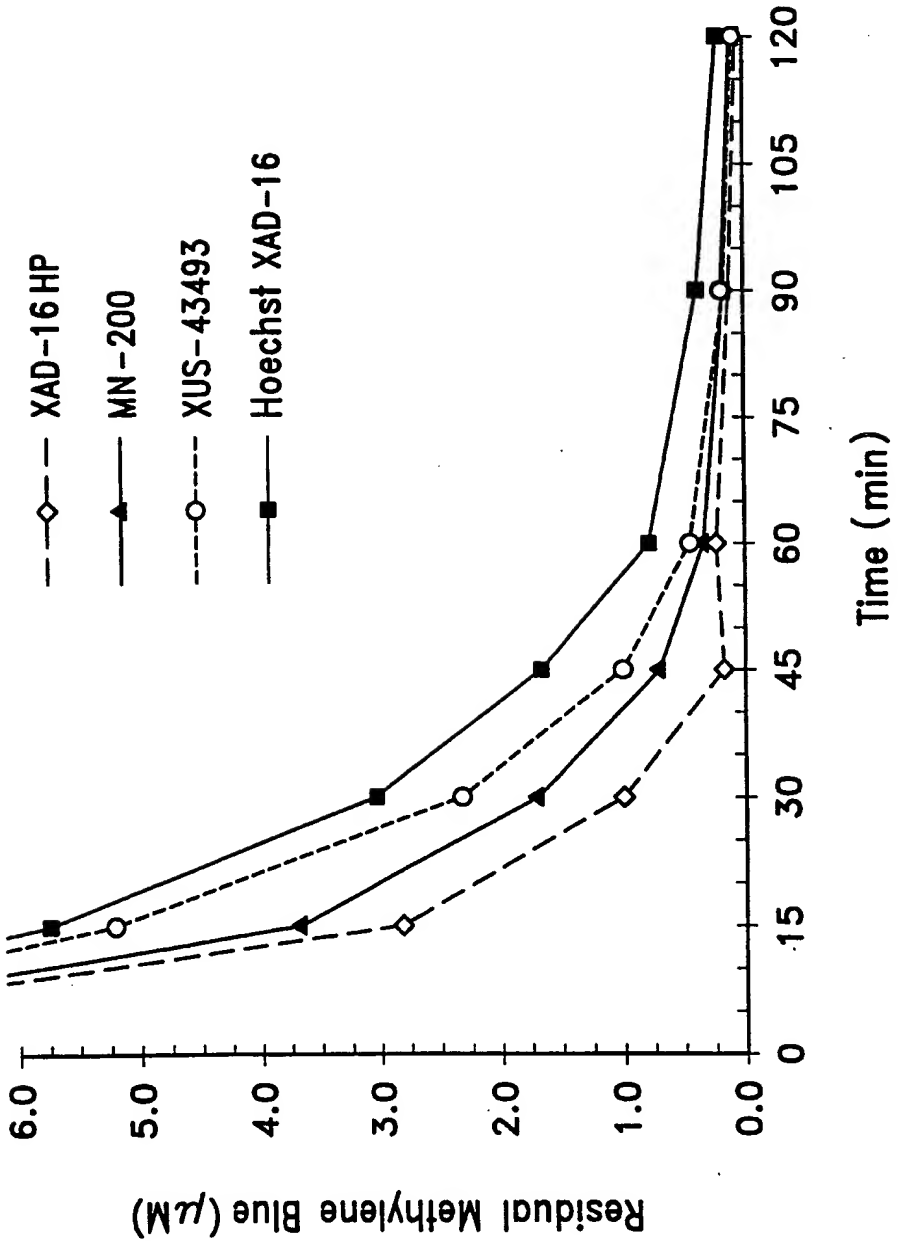
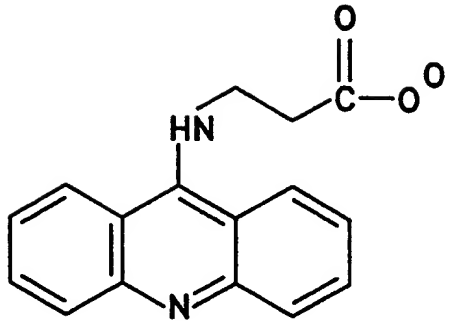
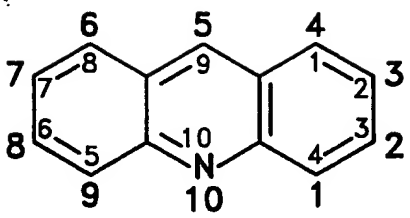

Residual S-59 (μM)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Time (hr)
Ethanol Pre-wet
No Tx
50% Glycerol
50% PEG-400

Fig. 10

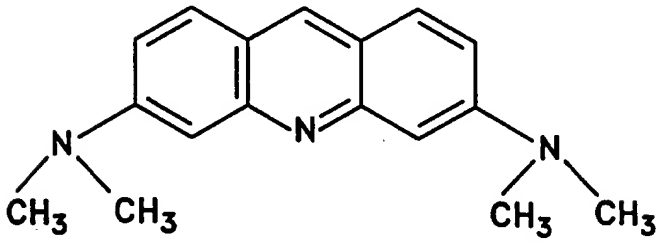

XAD-16 HP
MN-200
XUS-43493
Hoechst XAD-16
Residual Methylene Blue (μM)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Time (min)
0
15
30
45
60
75
90
105
120



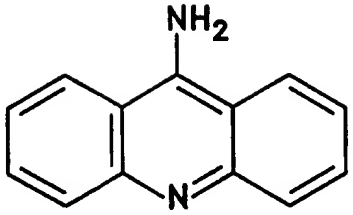

5-[(β-carboxyethyl) amino] acridine




Acridine




Acridine Orange




9-Amino Acridine

Fig. 11

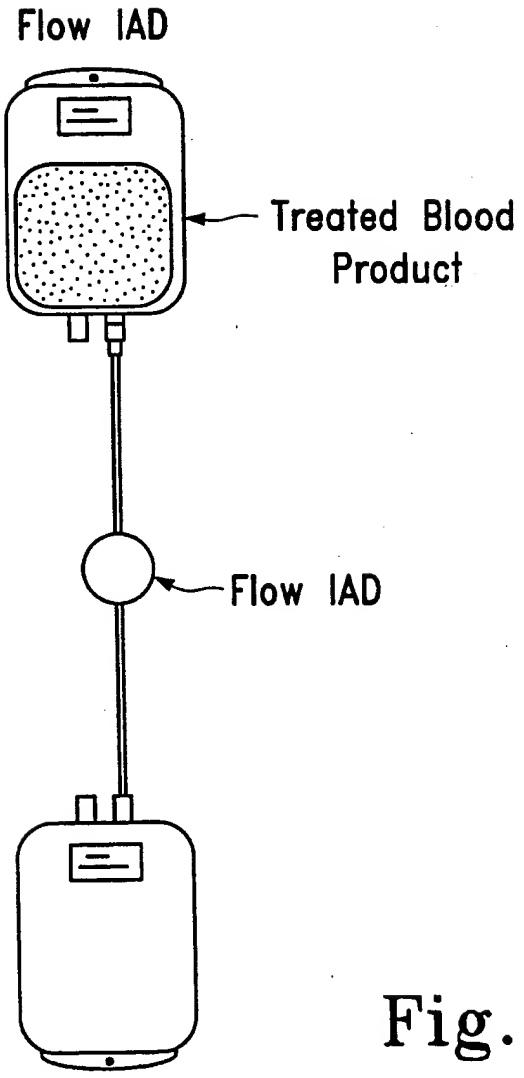



Fig. 12

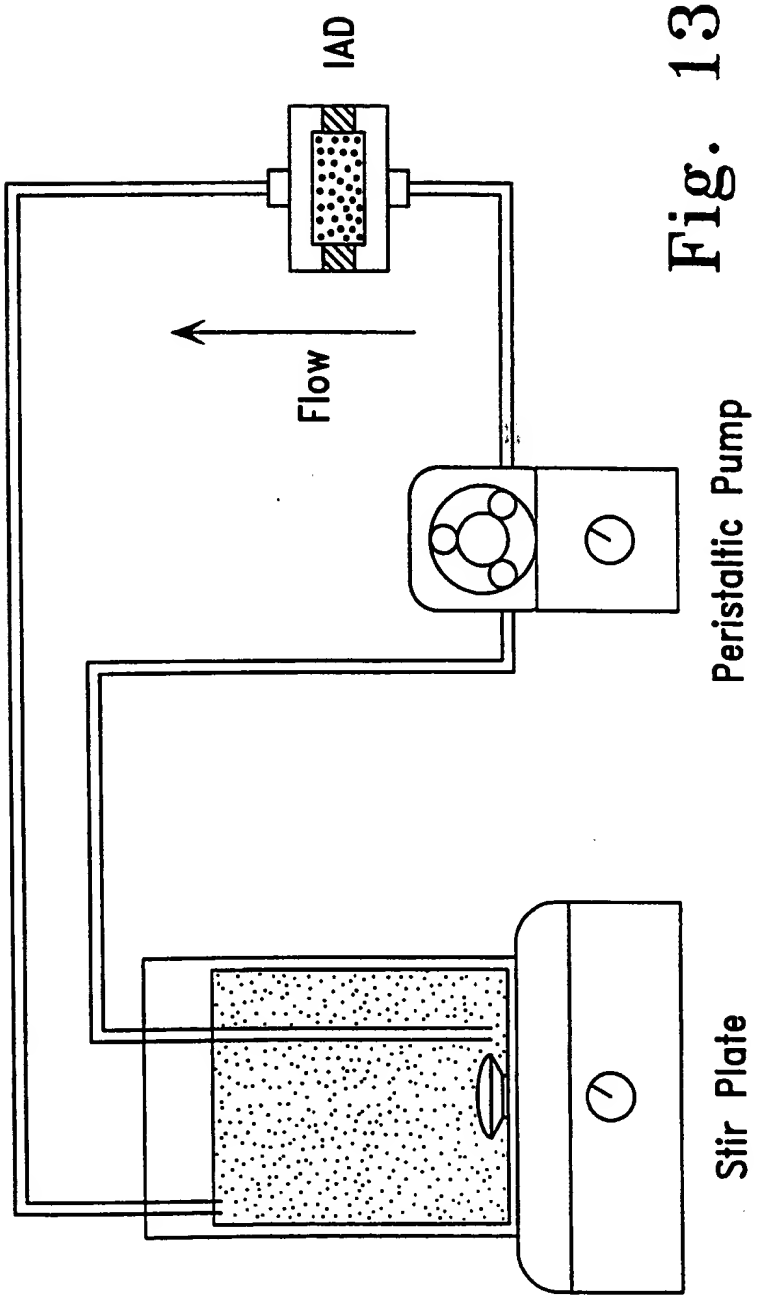
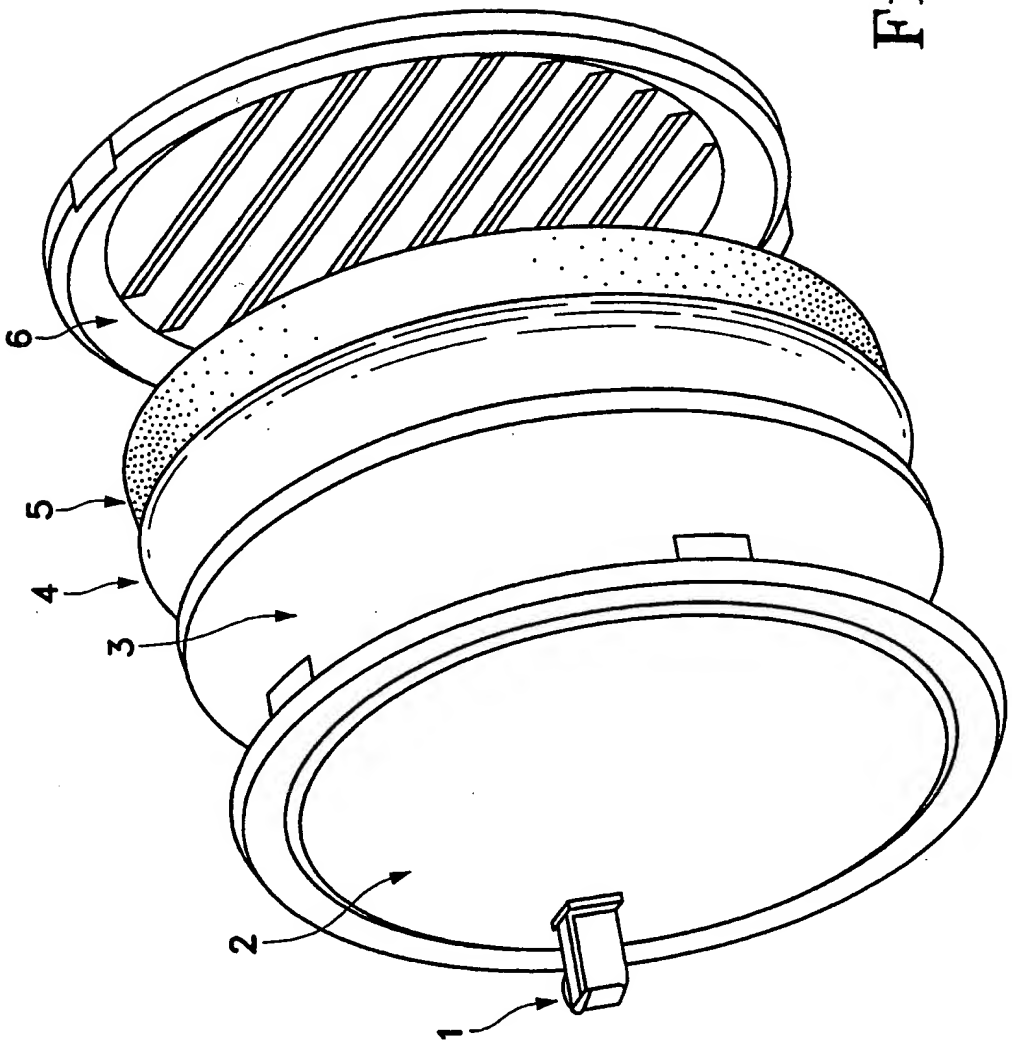



Fig. 13

Fig. 14



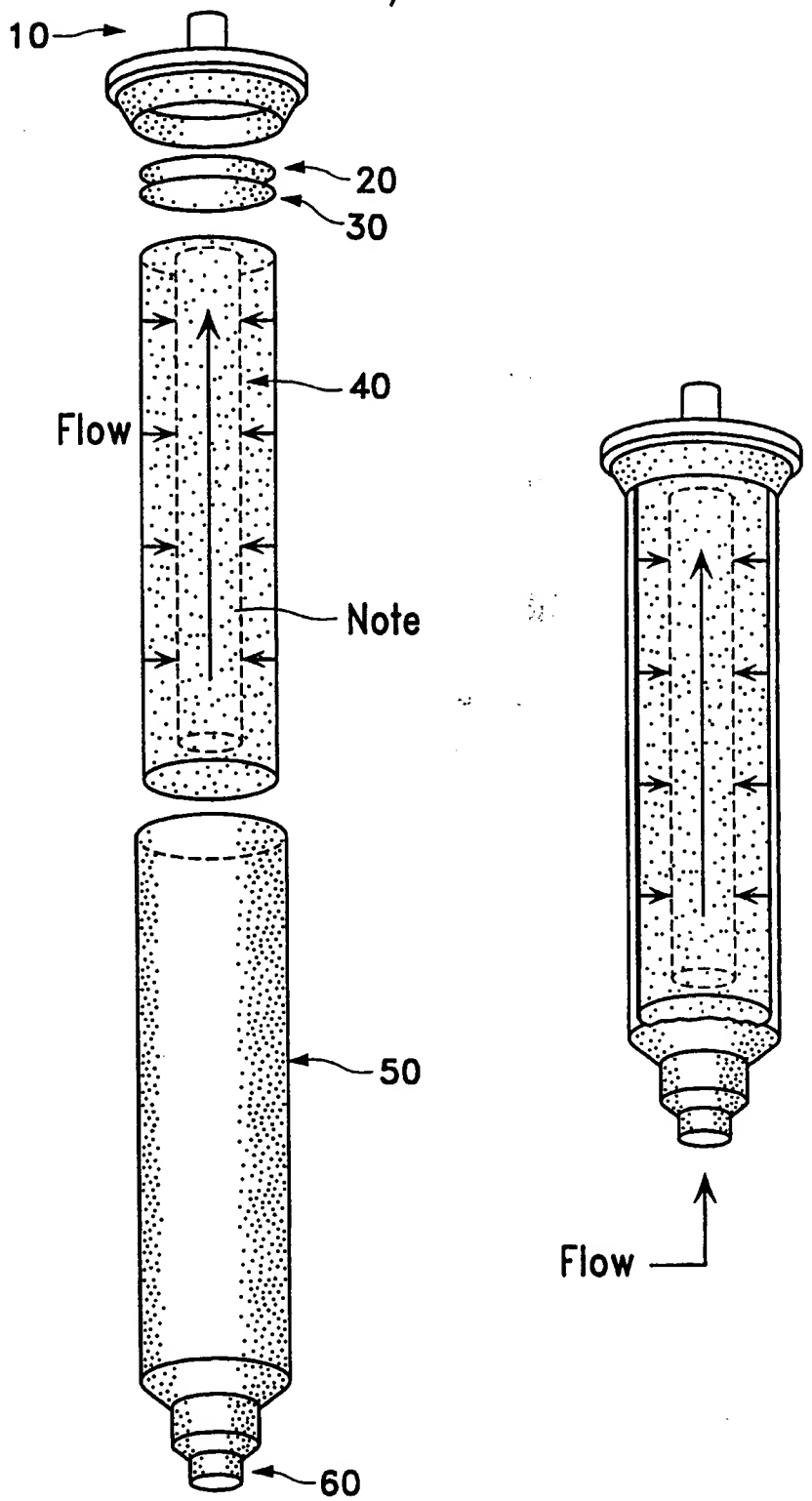



Fig. 15

# INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 98/14211

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01J20/28 A61L2/00 B01D15/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01J A61L B01D

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | US 5 147 722 A (KOSLOW) 15 September 1992<br>see column 34-35; claims 29-31<br>---<br>-/-- | 1,7 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

| Date of the actual completion of theinternational search | Date of mailing of the international search report |
|---|---|
| 15 October 1998 | 23/10/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Wendling, J-P |

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# INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 98/14211

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| E | WO 98 30327 A (CERUS CORP.) 16 July 1998<br>see page 111-114; claims 1-31<br>----- | 1,6,10 |

2

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Information on patent family members

Inte onal Application No
PCT/US 98/14211

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Request is:

1
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# cDNA-derived amino-acid sequence of lentil seedlings' amine oxidase

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Lentil seedlings' amine oxidase (LSAO) cDNAs were identified in a cDNA λgt 10 library by plaques hybridization. The nucleotide sequence of a 2111 bp clone was determined. It contains part of a signal peptide, the complete sequence coding for the mature protein and the 3'-untranslated region of the mRNA. The deduced protein sequence shows that the mature protein is composed of 569 amino acids with a molecular mass of 67 kDa, also taking into account the glucidic component. The LSAO cDNA was identified by sequencing the N-terminal part of the protein and several tryptic peptides. The protein sequence shows a characteristic hexapeptide present in amine oxidases containing 6-hydroxydopa as the organic cofactor. Three conserved histidines might be the ligands of copper bound to the enzyme.

6-Hydroxydopa; Amine oxidase; cDNA cloning; *Lens culinaris*

## 1. INTRODUCTION

Copper containing amine oxidases (AOs) (EC 1.4.3.6) catalyze the oxidative deamination of polyamines to the corresponding aldehydes with the production of ammonia and hydrogen peroxide. AOs are widespread among living organisms. Though the function of these enzymes still awaits to be elucidated, their action on polyamines grants an important physiological role. In fact polyamines are involved in a variety of processes in plants. Putrescine levels have been found to increase during ripening of fruits of a tomato cultivar with a prolonged storage life [1]. Accumulation of polyamines has been shown to induce chilling tolerance in maize [2], citrus [3] and zucchini squash [4]. A recent study shows that the most tolerant varieties of wheat to osmotic stress are those with the highest putrescine accumulation ability [5]. Putrescine accumulation is also involved in the response of plants to acid rain [6], low oxygen concentration [7] and to ozone treatment [8]. These examples show that accumulation of polyamines is a response to a great variety of stresses (see [9] for a review). Accumulation of polyamines is also observed during seedling development [10]. In lentils this accumulation is paralleled by an increase of amine oxidase activity [11] that reaches a peak when the seedlings are growing faster [12]. Despite the huge amount of studies conducted, little is known about the significance of these biochemical changes and the possible involvement of AOs.

Lentil seedlings' amine oxidase (LSAO) is a dimeric enzyme with a molecular weight of 140 kDa [13]. Each subunit contains a copper ion and an organic cofactor. The nature of the organic cofactor has been a matter of debate for long time. Recently it has been identified as 6-hydroxydopa (TOPA) in bovine serum amine oxidase (BSAO) [14]. Evidence has been presented for the presence of TOPA also in LSAO [15]. In this paper we describe the isolation and sequence analysis of the LSAO cDNA. An esapeptide presumably containing the TOPA cofactor was found as well as three sequences containing possible copper ligands.

*Abbreviations:* AO, amine oxidase; bp, base pair(s); BSAO, bovine serum amine oxidase; cDNA, DNA complementary to mRNA; Da, dalton; HPAO, *Hansenula polymorpha* amine oxidase; kDa, kilodalton; kb, kilobase (pair); LSAO, lentil seedlings' amine oxidase; nt, nucleotide(s); PTH, phenyl-thiohydantoin; SDS, sodium dodecyl sulphate; TOPA, 6-hydroxydopa.

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## 2. MATERIALS AND METHODS

### 2.1. *Plant material*

Lentil (*Lens culinaris*) seeds were soaked for 12 h in autoclaved tap water at room temperature. The seeds were then grown in a greenhouse at 25°C in the dark for 5 days and watered daily with tap water.

### 2.2. *Enzyme isolation and amino-acid sequence analysis of LSAO*

Amine oxidase was purified from lentil seedlings as described by Flores et al. [13].

LSAO (5 mg) was solubilized in 0.1 M ammonium bicarbonate pH 7.8 in the presence of 2 M urea and digested with trypsin (enzyme/LSAO ratio 1:50 (w/w)) at 37°C, overnight. Tryptic peptides were purified on a reverse-phase column (Aquapore RP 300, 4.6 × 250 mm, Applied Biosystems) developed in 60 min with a linear gradient of 0% to 60% acetonitrile in 0.2% trifluoroacetic acid, generated in a Beckman model 340 instrument at a flow rate of 1.0 ml/min. The elution of peptides was monitored both at 220 and 280 nm with a Beckman model 165 spectrophotometer.

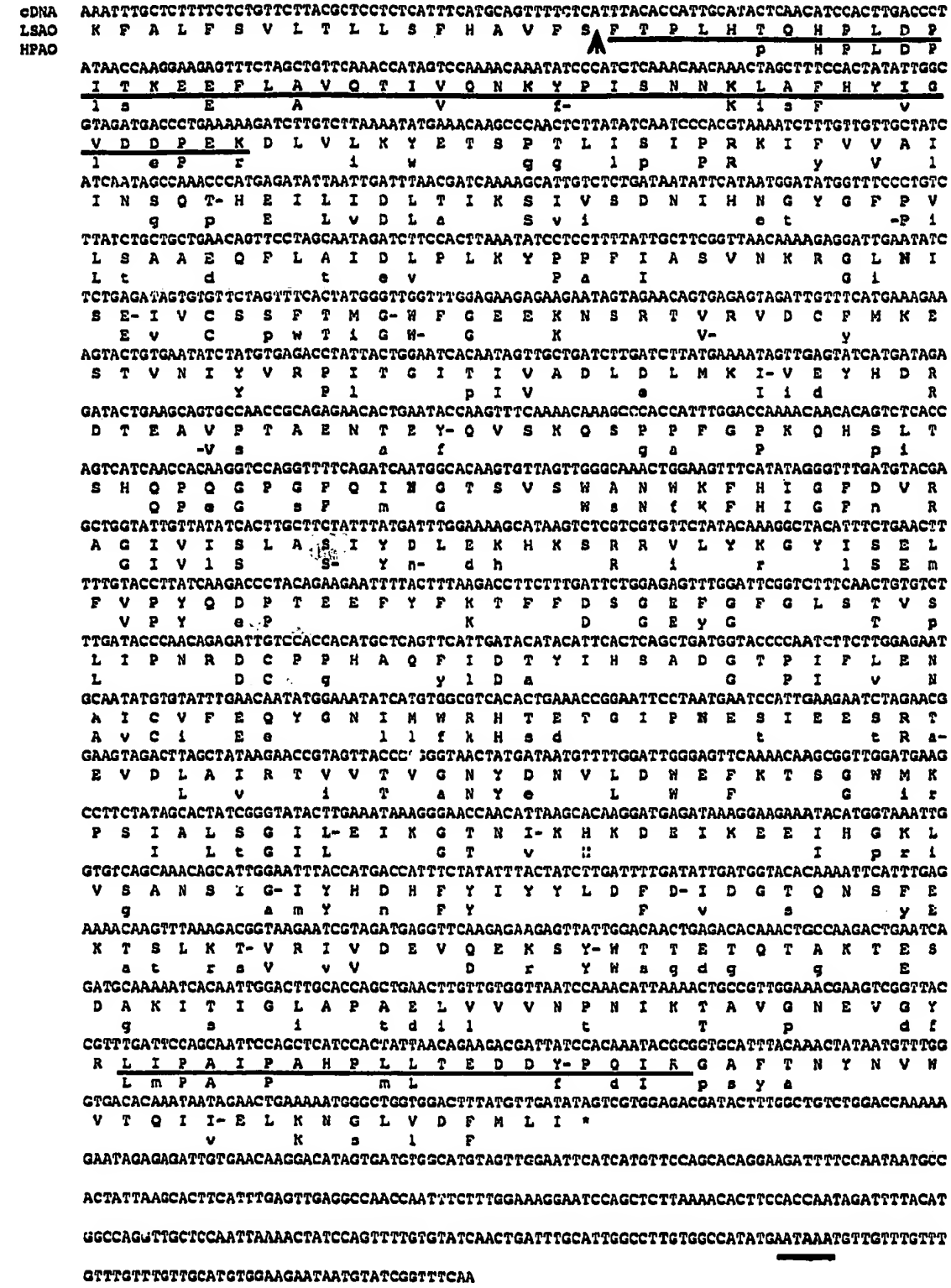

Fig. 1. Comparison of cDNA and the amino-acid sequence of LSAO with the sequence of yeast (*Hansenula polymorpha*) amine oxidase (HPAO) [24]. Numbering of the amino-acid sequence refers to the amino terminus of the mature protein. In the HPAO sequence only identical (upper case) or similar (lower case) residues to the LSAO sequence are shown. Hyphens indicate a gap introduced for maximizing matches. The features marked are: the cleavage site of a signal peptidase (vertical arrow), peptides directly sequenced by Edman degradation (underlined), possible glycosylation sites (asparagines in bold), the polyadenilation site (thick underlined).

Automated Edman degradation was carried out using an Applied Biosystems model 470A gas phase sequencer equipped with an Applied Biosystems model 120A PTH analyzer for the on-line detection of the PTH-amino acids.

2.3. *Construction and screening of the cDNA library*

Total RNA was extracted from 5-days-old lentil seedlings grown in the dark as described previously [16]. Polyadenylated RNAs were isolated by affinity chromatography on oligo(dT)-cellulose [17]. 10 g of seedlings yielded 2 mg of total RNA and 50 $\mu$g of poly(A)$^+$ RNA. The cDNA was synthesized using the Riboclone cDNA synthesis system (Promega) and tailored with *Eco*RI linkers using the Riboclone *Eco*RI linker ligation system (Promega). The yield of cDNA was 1 $\mu$g starting from 5 $\mu$g of poly(A)$^+$ RNA.

The tailored cDNA was then ligated into dephosphorylated, *Eco*RI cut, $\lambda$gt 10 and the resulting recombinant phage DNAs were packaged using the Packagene system (Promega). The recombinant phages were plated on *E. coli* C600 Hfl for screening. 100 ng of cDNA yielded 500,000 plaques. The library was screened by plaque hybridization [18] using end-labelled oligonucleotides with [$\gamma$-$^{32}$P]ATP (Amersham).

Oligonucleotides sequences were 5'-TGNGGRTARTCRTCYTC-3' and 5'-ATNGGRTAYTTKATKTG-3' (N=A,T,C, or G; R=A or G; Y=C or T; K=G or T). They were purchased from GENESET.

2.4. *DNA sequencing*

cDNAs to be sequenced were subcloned into pBS (Stratagene) and both strands were sequenced using the Sequinase version 2.0 kit (USB) applied to plasmid DNA [19]. Nested deletions were made with Erase-a-base system (Promega). The primers used for sequencing were the M13-20 primer present in the Sequinase kit and the reverse sequencing primer 5'-CAGGAAACAGCTATGAC-3' (Promega).

Since there are two *Eco*RI sites and since the library was made with *Eco*RI linkers the cDNA was also sequenced directly in $\lambda$gt 10 to confirm the sequences around the *Eco*RI sites. The possibility of having chimeric clones was excluded because the first *Eco*RI site is within the coding region of the protein, for which there is sequence information, and the second one does not contain the characteristic sequence of the linker.

2.5. *Sequence comparison and hydropathy analysis*

Conservative amino acid substitution was as reported in ref. [20].

Hydrophobicity values were calculated using a window size of 9 residues according to Kyte and Doolittle [21].

## 3. RESULTS

Total mRNA from 5-days-old lentil seedlings grown in the dark was taken to construct a cDNA library in the $\lambda$gt 10 vector. This is the richest source of mRNA coding for LSAO, as previously shown [12].

Two degenerated oligonucleotides representing DNA sequences complementary to all possible coding sequences for the tryptic peptides GluAspAspTyrProGln and GlnAsnLysTyrProIle were used to screen the library by plaque hybridization. Approximately 50,000 recombinant phages were screened, from which two positives clones were found with inserts of 2000 and 2100 bp, respectively. They were subcloned in pBS and subjected to sequence analysis. The longest insert contained the full coding region for the mature protein plus a short hydrophobic peptide besides the 3'-untranslated region of the LSAO mRNA.

The identity of the LSAO cDNA was established by the correspondence with the N-terminal sequence of

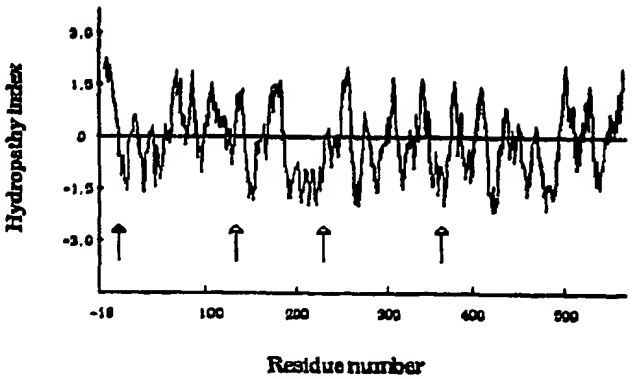


Fig. 2. Hydropathy plot of the LSAO amino-acid sequence derived from cDNA. The black-headed arrow indicates the N terminus of the mature protein. The white-headed arrows indicate three possible glycosylation sites. Numbering refers to the amino terminus of the mature enzyme. Hydrophobicity values were calculated using a window size of 9 residues according to Kyte and Doolittle [21]. Values indicating hydrophobic and hydrophilic regions are above and below zero, respectively.

LSAO protein and with several other tryptic peptides of the deduced amino acid sequence (Fig. 1). The open reading frame of 1707 nucleotides (Fig. 1) encodes a polypeptide of 569 amino acids with a molecular weight of 64,321 Da that, taking into account the presence of about 3% carbohydrates (G. D'Andrea, personal communication), gives a molecular mass of 67 kDa for the monomer, which is in agreement with the reported value of 70 kDa [13]. The amino-acid composition of the deduced protein sequence is similar to that determined from purified LSAO [13]. There are three putative glycosylation sites (Fig. 1) of which two only bear a carbohydrate chain (G. D'Andrea, personal communication). The sites at position 234 and 364 seem the most likely candidates since the other site appears to be located in a hydrophobic region (Fig. 2).

The deduced amino acid sequence of the largest insert does not contain methionines upstream the N-terminal residue (phenylalanine) of the mature protein, and therefore it is part of a supposed signal peptide which is incomplete in this clone. The cleavage site (Figs. 1 and 2) conforms to the "(−3,−1) rule" [22]. The deduced protein sequence shows a region of six highly conserved amino acids among copper containing AOs (Fig. 3) [23] where the tyrosine in the third position was found to be modified into TOPA in BSAO.

In an attempt to find out the ligands of the copper, homologies were searched for in already known sequences of copper-containing AOs. Fig. 1 shows three regions of significant similarity between LSAO and yeast AO [24]. They all contain a conserved histidine residue.

There is a 3'-untranslated sequence of 350 nucleotides besides the 1707 nucleotides of the open reading frame.

| Source | | Method | Ref. |
|---|---|---|---|
| Bovine serum | LNXDYV | Edman degradation | 14 |
| Porcine serum | LNXDYV | Edman degradation | 23 |
| Pig kidney | YNXDY | Edman degradation | 23 |
| Yeast | ANXEYC | Edman degradation | 23 |
| Yeast | ANYEYC | cDNA | 24 |
| Pea seedlings | GNXDNV | Edman degradation | 23 |
| Lentil seedlings | GNYDNV | cDNA | This work |

Fig. 3. Comparison of the amino-acid sequence around TOPA in several amine oxidases obtained either from cDNA sequencing or direct sequence of the purified enzymes. X indicates a blank position obtained by Edman degradation.

It contains the eucaryotic polyadenylation signal AATAAA (Fig. 1).

## 4. DISCUSSION

A previous study showed that lentil seedlings grown in the dark for five days are the best source of LSAO cDNA [12]. This was the starting material for the construction of a cDNA library in $\lambda$gt 10. Oligonucleotides constructed with reference to tryptic peptides were used to isolate LSAO cDNA clones by plaque hybridization. Sequencing the largest positive clone yielded an open reading frame of 1707 nucleotides and a 3'-untranslated region of 350 nucleotides. The latter contain a typical polyadenylation signal (Fig. 1).

The deduced protein sequence shows a putative signal peptide extending 18 residues upstream the N-terminal Phe and a peptidase cleavage site which was confirmed by sequencing the N-terminal part of the protein. There are no methionines present in this peptide, hence the sequence of the presumptive signal peptide appears to be incomplete.

The N-terminal sequence, together with that of 4 other tryptic peptides (Fig. 1) gave direct proof on the identity of this cDNA with LSAO. The predicted sequence of the mature protein shows that the monomer is composed of 569 amino acids with a calculated molecular weight of 67 kDa, in good agreement with a previous report [13]. It also shows three putative glucosylation sites. LSAO is in fact a glycoprotein containing two carbohydrate chains per monomer (G. D'Andrea, personal communication), therefore only two of these sites actually bind carbohydrates. Inspection of the hydropathy plot in Fig. 2 suggests that Asn-234 and Asn-364 may be *N*-glycosylated, since Asn-130 sits in a hydrophobic environment.

Copper-containing AOs have two different cofactors. One is cupric copper and the other has been recently identified as TOPA [14] which most probably results from a post-synthetic modification of a tyrosine. The amino-acid sequence of LSAO shown in Fig. 3 is very similar to the active-site peptide of bovine serum AO, where the presence of TOPA has been demonstrated at the position X [14]. This sequence is also present in other AOs where the presence of TOPA has been inferred from resonance Raman studies [23,25]. Therefore this sequence could represent a consensus sequence for TOPA quinone, which can be formed from the tyrosine located between an asparagine and an aspartic acid as proposed previously [23]. It must also be pointed out that recent EPR evidence has suggested the presence of TOPA quinone in LSAO [15]. From all this data it can be inferred that the tyrosine at position 387 of LSAO cDNA is modified to TOPA in the mature protein.

A sequence comparison between yeast AO [24] and LSAO was carried out in order to find out possible ligands for the copper. The similarities between these two proteins besides the region of the organic cofactor lie in three positions only, as shown in Fig. 1. All these three sequences contain a histidine residue. Previous spectroscopic studies have shown that the copper ion in BSAO should have three histidines coordinated [26]. Therefore the histidines at positions 8, 246 and 357 can be reasonably proposed as copper ligands.

Expression of active LSAO in vivo and site-directed mutagenesis will demonstrate if the histidines 8, 246 and 357 are actually the copper ligands. The importance of individual amino acids for the possible post-translational modification leading to the appearance of 6-hydroxydopa will be also assessed.

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*Note added in proof*

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDJB Nucleotide Sequence Databases under the accession number X64201.